Synergistic Nanozymetic Activity of Hybrid Gold Bipyramid–Molybdenum Disulfide Core@Shell Nanostructures for Two-Photon Imaging and Anticancer Therapy

Swarup Kumar Maji,†,‡§ Subin Yu,† Kyungwha Chung,†,‡# Madeshwaran Sekkarapatti Ramasamy,† Ju Won Lim,† Jianfang Wang,§# Hyukjin Lee,†¶ and Dong Ha Kim*†,‡,⊥

†Department of Chemistry and Nano Science, Division of Molecular and Life Sciences, College of Natural Sciences, Ewha Womans University, Seoul 03760, Korea
‡Department of Chemistry, Khatra Adibasi Mahavidyalaya, Khatra 722140, West Bengal, India
§Department of Physics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China
¶College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Korea
⊥State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China

ABSTRACT: In recent years, the concept of combined therapy using gold hybrid nanomaterials has been broadly adopted to pioneer new anticancer treatments. However, their synergistic anticancer effects have yet to be thoroughly investigated. Herein, a hybrid gold nanobipyramid nanostructure coated with molybdenum disulfide (MoS2) semiconductor (AuNBPs@MoS2) was proposed as a smart nanozyme for anticancer therapy and two-photon bioimaging. The hybrid material showed dramatically enhanced localized surface plasmon resonance property under excitation owing to its anisotropic nature, coupled with the rich electron density in MoS2, resulting in the superior in situ photogeneration of reactive oxidative species (ROS -1O2, •OH). We demonstrated that the synergistic effect of enhanced photothermal conversion and generation of ROS could increase the anticancer effect of AuNBPs@MoS2. Two-photon luminescence imaging confirmed that AuNBPs@MoS2 was successfully internalized in cancer cells and that simultaneous anticancer treatments based on catalytic and photothermal therapy could be achieved. This study highlighted, for the first time, a novel approach of plasmon-mediated powerful anticancer therapy and imaging via the unprecedented combination of anisotropic AuNBPs and two-dimensional MoS2 material.

KEYWORDS: localized surface plasmon resonance, gold nanobipyramid, photodynamic therapy, photothermal therapy, two-photon imaging

INTRODUCTION

Over the past few years, molybdenum disulfide (MoS2), a two-dimensional (2D) transition-metal dichalcogenide nanomaterial, has attracted distinct attention beyond graphene, because of its unique optical, electronic, and mechanical properties.1–3 Two-dimensional MoS2 nanosheets (NSs) have shown significant promise in a wide range of fields including electronic devices, transistors, energy storage devices, and catalysis.4–6 Very recently, 2D MoS2 nanosheets have also come to be considered as an emerging class of nanomaterials for various biomedical applications, such as DNA biosensors, blood glucose detection, antibacterial agents, NIR photothermal agents, and drug delivery with excellent biocompatibility and low toxicity in living organisms.17–19 In addition, the MoS2 NSs could also perform as artificial enzymes, with so-called peroxidase-like activity (POD) for the highly sensitive and selective colorimetric detection for H2O2 and glucose in serum.11–14 In order to further achieve improved performance through synergistic effects, attempts have been made to decorate/combine MoS2 NSs with other effective materials, such as metal chalcogenides,15 carbon nanomaterials,16 metal oxides,17 noble metals18 and so on. Between all of these techniques, the decoration of metal nanostructures (Au, Ag, and Pt) with their unique surface plasmon resonances to the semiconductor MoS2 NSs are considered to be impressive candidates for hybrid nanostructures.20 Over the past several decades, gold nanocrystals (Au NCs) have been investigated extensively due to their pronounced prospective applications in...
biomedical science, catalysis, photonics, and electronics in which most of these applications are on the basis of their fascinating LSPR features. To date, numerous shapes of NCs such as nanospheres, nanorods (NRs), nanocages, and nanostars have already been employed in biological fields because of their tunable LSPR properties through alteration of their shapes and sizes. However, in comparison to isotopic Au NCs, anisotropic NCs offer more noteworthy benefits due to the presence of two plasmon modes, transverse mode and longitudinal mode. The fascinating longitudinal LSPR can easily be tailored from 580 to 1200 nm by tuning their aspect ratios to cover the range from the visible to NIR region. Although the excellent LSPR properties of anisotropic Au NCs have been successfully utilized for many cutting-edge applications, they still have some deficiencies. The synthesized Au NRs have larger size distributions, and thus cause the broadened longitudinal LSPR with full width at half-maximum values in the range of about 150 to 200 nm. Moreover, the round ends of Au NRs exhibit a relatively less localized electric field enhancement. Recently, Au NBPs, which are composed of two pyramids connected at their bases, have been invented as another class of diverse anisotropic nanostructures which simultaneously possess synthetically tunable longitudinal LSPRs with sharp tips. The narrower full width at half-maximum, along with the enhancements of localized electric field at rates several times higher than Au NRs, make Au NBPs much more sensitive toward the surrounding environments.

In this work, we have designed a core@shell like structure of nanohybrid material composed of Au NBPs wrapped with MoS2 NSs (AuNBPs@MoS2). The nanohybrids have shown enhanced and synergistic performance as an artificial nanozyme through the oxidation of a typical peroxidase substrate TMB (3,3′,5,5′-tetramethylbenzidine) in the presence of H2O2 and under LSPR excitation. This peroxidase property, along with excellent NIR absorbance property by both Au NBPs and MoS2, made the hybrid an effective therapeutic agent for cancer treatment by catalytic therapy (CLT) and photothermal therapy (PTT). In addition, the in vitro cellular imaging capability was also investigated due to the eminent two-photon luminescence (TPL) of Au NBPs.

**RESULTS AND DISCUSSION**

The Au NBPs was first synthesized, then, CTAB (hexadecyltrimethylammonium bromide)-capped Au NBPs were further modified with APTES ((3-aminopropyl) triethoxysilane) in order to graft more positive charges on the surface. The core@shell like hybrid was obtained through the electrostatic interaction of positively charged Au NBPs with the negatively charged MoS2 NSs (see Supporting Information for more details). As observed from the field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) images (Figure S1b,f), the average length and width of the Au NBPs were about 110 and 36 nm, respectively. After wrapping with MoS2 NSs (Figure S1a,e), a thin layer of 1.5 to 2 nm could be observed on the surface of Au NBPs, as displayed by the FESEM (Figure 1a), TEM (Figure 1b), and high-resolution TEM (HRTEM) (Figure 1c) images. The transmission electron microscopy energy-dispersive X-ray spectroscopy (TEM-EDX) elemental mapping was conducted (Figure 1d–h), and the symmetrical
respectively. In the case of AuNBPs@MoS2, the E2g peak of AuNBPs@MoS2 exhibited comparable absorption. MoS2 demonstrated the strong characteristic peaks in the near-IR region, while Au NBPs showed a strong LSPR band at 791 nm with a weak transversal band at 515 nm (Figure 1k). The UV−vis spectrum of ADBA in 0.1 M acetate buffer (pH = 4.5) reacted with (i) H2O2, (ii) AuNBPs@MoS2, (iii) AuNBPs, and H2O2, (iv) AuNBPs@MoS2 and H2O2 followed by irradiated with NIR light for 1 h, and (v) AuNBPs@MoS2, followed by irradiated with NIR light for 1 h. (d) UV−vis absorbance spectra of ADBA in 0.1 M acetate buffer in the presence of AuNBPs@MoS2 under 808 nm NIR light illumination (CW, 2 W/cm2) for 4 h. (e) Photothermal evolution curves of (i) water, (ii) MoS2, (iii) Au NBPs, and (iv) AuNBPs@MoS2 over a period of 10 min exposure of NIR laser (808 nm, CW, 2 W/cm2). (f) FDTD simulations and local field enhancement intensity of the (i) Au NBPs, and (ii) AuNBPs@MoS2 obtained under incident excitation wavelength of 808 nm.

The intrinsic artificial peroxidase-like property (POD) (i.e., nanozyme activity) of AuNBPs@MoS2 was explored by considering the oxidation of a typical peroxidase substrate TMB in the presence/absence of H2O2 using the colorimetric assays technique.14 In this case, the TMB was oxidized to a blue color product (charge-transfer complex resulting from one-electron oxidation of TMB, oxTMB) (Figure 2a (iv) by the catalyst (AuNBPs@MoS2) in the presence of H2O2, which could also be quenched by adding H2SO4 so as to generate the yellow color solution (Figure 2a, vii). This indicates that the presence of three components, viz. TMB, H2O2 and AuNBPs@MoS2 is essential to observe the normal POD reaction.11 In the case of a POD reaction, the colorless TMB was oxidized to a blue color product (charge transfer complex) further proving the interaction between Au NBPs and MoS2. The color reaction was monitored by the generation of two characteristic peaks for ox-TMB at 370 and 652 nm, respectively (Figure 2b). This phenomenon is quite similar to that of the natural enzyme horseradish peroxidase (HRP). Moreover, the oxidation of TMB was not significantly observed in the case of the reaction conditions (i) TMB with H2O2, (ii) AuNBPs@MoS2 with TMB, and (iii) AuNBPs@MoS2 without H2O2 (Figure 2a), and no obvious blue color generation was observed in these cases either (Figure 2b(i−iii)). This indicates that the presence of component AuNBPs@MoS2 is essential to observe the normal POD reaction.9,11,14,29 Now, in contrast with the normal POD condition, the blue color generation reaction (in the presence of TMB, H2O2 and AuNBPs@MoS2) was further conducted under the excitation of LSPR of the hybrid (808 nm NIR light, contentious wave), and a dramatic enhancement in POD activity of about 2-fold was obtained by the generation of more intense peaks at 652 nm (Figure 2a(v), and b(v)).20,30 Again, in contrast to the normal POD reactions, we further tested the POD activity beyond the normal condition, in which contact between catalyst, TMB, and H2O2 is essential.11,14,29 In this case, the TMB was reacted with AuNBPs@MoS2 under NIR light illumination (absence of H2O2), and a significant blue color product was observed (Figure 2a(vi), and b(vi)), although it was not efficient enough to compete with normal.

Figure 2. Nanozymatic and photothermal property of AuNBPs@MoS2. (a) Digital photographs and (b) UV−vis absorbance spectra of TMB-H2O2 solution system in the presence/absence of AuNBPs@MoS2 under varying reaction parameters. Reaction conditions: 0.1 M acetate buffer, pH 4.5, ∼37 °C, NIR light −808 nm, CW, 2 W/cm2, 30 min. (c) Photoluminescence spectra of TA solution in 0.1 M acetate buffer (pH = 4.5) reacted with (i) H2O2, (ii) AuNBPs@MoS2, (iii) AuNBPs@MoS2, and H2O2, (iv) AuNBPs@MoS2 and H2O2 followed by irradiated with NIR light for 1 h, and (v) AuNBPs@MoS2, followed by irradiated with NIR light for 1 h. (d) UV−vis absorbance spectra of ADBA in 0.1 M acetate buffer in the presence of AuNBPs@MoS2 under 808 nm NIR light illumination (CW, 2 W/cm2) for 4 h. (e) Photothermal evolution curves of (i) water, (ii) MoS2, (iii) Au NBPs, and (iv) AuNBPs@MoS2 over a period of 10 min exposure of NIR laser (808 nm, CW, 2 W/cm2). (f) FDTD simulations and local field enhancement intensity of the (i) Au NBPs, and (ii) AuNBPs@MoS2 obtained under incident excitation wavelength of 808 nm.
POD reaction and under NIR illumination in the presence of H$_2$O$_2$; however, they can easily contribute to the one another in order to increase the activity. Therefore, we have successfully evaluated that the AuNBPs@MoS$_2$ hybrid has multiple nanozymetic properties, which could be further enhanced under LSPR excitation.

The steady state kinetics were further investigated by the initial rate method in order to determine the kinetic parameters and also to better understand the POD activity of AuNBPs@MoS$_2$ under LSPR illumination. Typical Michaelis–Menten curves were achieved for the oxidation reactions within a varying concentration range of TMB and H$_2$O$_2$. The kinetic parameters were obtained by calculating the apparent Michaelis–Menten constant ($K_m$) and maximum reaction velocity ($V_{max}$). The apparent $K_m$ value for TMB as substrate was significantly lower than that of HRP and MoS$_2$, indicating that the AuNBPs@MoS$_2$ has higher POD activity toward TMB than that of HRP and MoS$_2$. Meanwhile, the apparent $K_m$ value for H$_2$O$_2$ as substrate was largely decreased compared to that of HRP, further indicating that AuNBPs@MoS$_2$ has higher catalytic activity toward H$_2$O$_2$ than HRP. The significantly lower $K_m$ values and higher $V_{max}$ values toward TMB and H$_2$O$_2$ compared to HRP, as well as some recent reports on MoS$_2$, suggest the synergistic and enhanced POD activity of the AuNBPs@MoS$_2$ under NIR light illumination. The mechanism of superior POD performance is ascribed by the several redox steps for the oxidation of TMB in the presence of H$_2$O$_2$, and the decomposition of H$_2$O$_2$ to hydroxyl radical ($\cdot$OH) in acidic conditions and thus improw the reaction rate of TMB oxidation.

Moreover, under LSPR excitation the oxidation of TMB was remarkably promoted due to the plasmonic effect and generation of hot electrons on Au NBPs, and the decomposition of H$_2$O$_2$ to hydroxyl radical ($\cdot$OH) in acidic conditions and thus improw the reaction rate of TMB oxidation. The improved EM properties using an orthogonal approach, we performed the rate of POD reaction and under NIR illumination in the presence of H$_2$O$_2$. In contrast, the generation of another ROS responsible for photocatalytic events, singlet oxygen ($^1O_2$) was also detected using 9,10-anthracenediyl- bis(methylene) dimalonic acid (ABDA) as a molecular probe (Figure 2d and S3). In this case, ABDA was reacted with $^1O_2$ to form an endoperoxide, thus causing a decrease in the absorption intensity of the anthracene core of ABDA. The time-dependent reduction in the absorbance of ABDA in the presence of AuNBPs@MoS$_2$ under NIR light illumination (without H$_2$O$_2$) (Figure 2d) further suggests the in situ generation of $^1O_2$ from dissolved O$_2$ and this $^1O_2$ could also contribute to the conversion to $\cdot$OH in order to further enhance the catalytic activity. Therefore, the phenomenon of the superior and multiple nanozymetic properties of AuNBPs@MoS$_2$ under NIR light illumination was achieved due to the enhanced catalytic generation of ROS from H$_2$O$_2$, H$_2$O and O$_2$.

The photothermal property of AuNBPs@MoS$_2$ under NIR irradiation (808 nm, CW, 2 W/cm$^2$) was then explored in terms of utilizing the hybrid material for cancer therapy. As shown in the photothermal heating curve (Figure 2e(iv)), a significant temperature increment was observed for AuNBPs@MoS$_2$ (100 $\mu$g/mL) under NIR light illumination. The rate of temperature increase was much more pronounced within the initial 3 min and reached a maximum of 60.3 °C after 10 min of laser irradiation. In the case of pure water (Figure 2e(i)), a negligible temperature increment was recorded, whereas MoS$_2$ and Au NBPs showed maximum temperature increments up to 50.6 and 55.6°C, respectively (Figure 2e(ii,iii)). Further assessment for the photothermal transduction ability of AuNBPs@MoS$_2$ was conducted by the solution containing AuNBPs@MoS$_2$ under exposure of three successive NIR laser (2 W/cm$^2$) on for 10 min and turned off for 15 min. As shown in Figure S4a, after three cycles of laser on/off, no significant loss of temperature was observed, proving the reproducibility and thermal stability of AuNBPs@MoS$_2$. The UV–vis absorbance spectrum and TEM image were also taken in order to examine the stability of AuNBPs@MoS$_2$ following laser irradiation, and no significant changes were detected in either of the cases (Figure S4b,c), however, the surface morphology was largely affected for bare Au NBPs (Figure S4d). The obtained photothermal effect of AuNBPs@MoS$_2$ nanohybrid was sufficient enough to promote thermal damage of the targeted tissue and could make it a promising candidate as a photothermal agent. In order to support our experimental results of enhanced light absorbance and photothermal properties using an orthogonal approach, we performed finite-difference time-domain (FDTD) simulation methods to calculate the electromagnetic (EM) field distribution and enhancement. As shown in Figure 2f, the EM fields were strongly distributed at the ends of both tips of Au NBPs, and the maximum peak intensity of the enhanced EM fields was increased from 19587.9 to 24969.7 after the MoS$_2$ wrapping on Au NBPs (AuNBPs@MoS$_2$). The improved EM field intensity by $\sim$21% for AuNBPs@MoS$_2$ further highlights the importance of efficient light absorption properties for obtaining an enhanced photothermal effect (0–20%).

The evaluation of the above-mentioned two interesting properties (peroxidase-like/nanozymatic/catalytic activity and photothermal property) that were then employed for in vitro therapeutics and imaging of cervical cancer (HeLa cells) for further applications in the biomedical field. For biological studies, the surface functionalization of AuNBPs@MoS$_2$ was
respectively (Figure 3a,i, orange bars). This unusual trend was noticed in which the cell viability at 50 μg/mL was 88.9%, whereas the values were 56.9 and 26.9% at 75 and 100 μg/mL, respectively (Figure 3a,ii). The reason behind this phenomenon was that AuNBPs@MoS2 could react with O2 and H2O2 (overexpressed in cancer cells) and enhance therapeutic efficiency, thus inducing cell death. In order to achieve multiple and enhanced therapeutic efficiency, we studied the cell survival under different conditions, shown in Figure 3a,ii. The cell survival study was done by first exposing the HeLa cells with AuNBPs@MoS2−PEG (50.0 μg/mL) for 12 h for effective endocytosis and then incubated with 100 μM of H2O2 for another 4 h. As displayed in Figure 3a,ii the MTT assay result showed that AuNBPs@MoS2−PEG could boost the H2O2 induced cancer cell damage of about 4% (yellow bars) compared to that of the control dose of AuNBPs@MoS2−PEG without H2O2 (88.9%). Interestingly, H2O2 (100 μM) alone and that with NIR illumination (blue bars) were unable to generate significant cell damage, perhaps due to the low concentration for enough generation of oxidative stresses inside the cells. However, with AuNBPs@MoS2−PEG the cytotoxicity was sufficient to overcome the barrier of oxidative stress and other factors for cell cytotoxicity, so we can speculate that the toxic potential was likely because of the POD property (absence of NIR light) of AuNBPs@MoS2−PEG with H2O2.15

In contrast, similar cell damage phenomenon could also be obtained by the use of MoS2 NSs with H2O2 (Figure 3a,ii, deep green bar), because MoS2 NSs are inherently cell cytotoxicity of AuNBPs@MoS2−PEG was then measured by the MTT assay method with HeLa cells after 12 h incubation and the concentration-dependent cell survival was noticed in which the cell viability at 50 μg/mL was 88.9%, whereas the values were 56.9 and 26.9% at 75 and 100 μg/mL, respectively (Figure 3a,ii). This unusual trend signifies that AuNBPs@MoS2 with POD activity denoted the apparent anticancer activity for catalytic cancer therapy (CLT). The reason behind this phenomenon was that AuNBPs@MoS2 could react with O2 and H2O2 (overexpressed in cancer cells) in the cell environment in order to generate highly toxic ROS, thus inducing cell death. In order to achieve multiple and enhanced therapeutic efficiency, we studied the cell survival under different conditions, shown in Figure 3a,ii. The cell survival study was done by first exposing the HeLa cells with AuNBPs@MoS2−PEG (50.0 μg/mL) for 12 h for effective endocytosis and then incubated with 100 μM of H2O2 for another 4 h. As displayed in Figure 3a,ii the MTT assay result showed that AuNBPs@MoS2−PEG could boost the H2O2 induced cancer cell damage of about 4% (yellow bars) compared to that of the control dose of AuNBPs@MoS2−PEG without H2O2 (88.9%). Interestingly, H2O2 (100 μM) alone and that with NIR illumination (blue bars) were unable to generate significant cell damage, perhaps due to the

made by SH-PEG in order to enhance the biocompatibility and stability in physiological condition.1 Because peroxidase is one of the important classes of natural enzymes, which are involved in controlling cellular redox homeostasis,32 it was then interesting to see in which way AuNBPs@MoS2−PEG act in cancer cells as well as their relationship with cytotoxicity. The inherent cell cytotoxicity of AuNBPs@MoS2−PEG was then measured by the MTT assay method with HeLa cells after 12 h incubation and the concentration-dependent cell survival was noticed in which the cell viability at 50 μg/mL was 88.9%, whereas the values were 56.9 and 26.9% at 75 and 100 μg/mL, respectively (Figure 3a,ii). This unusual trend signifies that AuNBPs@MoS2 with POD activity denoted the apparent anticancer activity for catalytic cancer therapy (CLT). The reason behind this phenomenon was that AuNBPs@MoS2 could react with O2 and H2O2 (overexpressed in cancer cells) in the cell environment in order to generate highly toxic ROS, thus inducing cell death. In order to achieve multiple and enhanced therapeutic efficiency, we studied the cell survival under different conditions, shown in Figure 3a,ii. The cell survival study was done by first exposing the HeLa cells with AuNBPs@MoS2−PEG (50.0 μg/mL) for 12 h for effective endocytosis and then incubated with 100 μM of H2O2 for another 4 h. As displayed in Figure 3a,ii the MTT assay result showed that AuNBPs@MoS2−PEG could boost the H2O2 induced cancer cell damage of about 4% (yellow bars) compared to that of the control dose of AuNBPs@MoS2−PEG without H2O2 (88.9%). Interestingly, H2O2 (100 μM) alone and that with NIR illumination (blue bars) were unable to generate significant cell damage, perhaps due to the
MoS2 using a multiphoton microscope by incubating the AuNBPs@MoS2@PEG with HeLa cells for detection and bioimaging purposes. The two-photon luminescence microscopy has the advantage of minimal photodamage and less background signals production with excellent three-dimensional in-depth image resolution formation compared to that of conventional fluorescence imaging technique. It has also been well studied that the sharp geometric features for Au NCs are required for greater field enhancement and subsequent intense TPL, therefore in this case Au NBPs with two sharp tips are one of the best candidates among the Au NC family. Therefore, we have studied the in vitro bioimaging capability of AuNBPs@MoS2−PEG, and as shown in Figure 4 a bright red color generation was observed in the cell cytoplasm after incubation for 12 h (Figure 4d−f) compared to that of control experiment (blank control and incubated with MoS2, Figure 4a,b), supporting the biomarking property of the hybrid material. Moreover, the HeLa cell incubated with AuNBPs also showed well distinguishable red fluorescence inside the cell cytoplasm (Figure 4c) due to the two-photon activity as mentioned above. Thus, the synergistic therapeutic and inherent imaging capability could make the AuNBPs@MoS2 as a valuable hybrid material for tumor detection and treatment in biomedical fields.

## Conclusion

In conclusion, we have for the first time designed a novel nanohybrid material by wrapping MoS2 NSs on anisotropic Au NBPs (AuNBPs@MoS2) with a length and width of 110 and 36 nm, respectively, which was exclusively characterized by HRTEM, FESEM, and EDX techniques. An obvious enhancement in optical absorbance and consequent photothermal activity (25 to 60.3 °C) was achieved due to the strong electronic interaction and plasmonic coupling between Au NBPs and MoS2 NSs. The AuNBPs@MoS2 hybrid showed superior peroxidase-like/nanozymatic activity in acidic conditions and the performance was further enhanced 2-fold with NIR light illumination, in which the generation of ROS was promoted through plasmonic effects. The potentiality of AuNBPs@MoS2 for subcellular localization was obtained by in vitro cellular uptake in HeLa cells and an intense two-photon luminescence image was captured. The explored catalytic and photothermal properties were then applied in order to achieve maximum therapeutic efficiency for HeLa cancer cells (13.2% cells were viable) in terms of sequential and/or combinational effects. In sum, an intense plasmonic coupling was observed by fabricating a new class of hybrid nanostructure, and the potentiality for detection and therapeutic effect was demonstrated for tumor treatment in vitro, which may serve as a basis for effective in vivo applications.

## Experimental Methods

**Chemicals.** Gold(III) chloride trihydrate (HAuCl4·3H2O; ≥99.9% trace metals basis), trisodium citrate dihydrate, sodium borohydride (NaBH4 99%), hexadecyltrimethylammonium bromide (CTAB, ≥98%), silver nitrate (AgNO3 ≥99.0%), ascorbic acid (AA), hexadecyltrimethylammonium chloride (CTAC, ≥98.0%), 3′-aminopropyl)triethoxysilane (APTES, 99%), 3,3′,5,5′-tetramethylbenzidine (TMB, ≥99.0%), 9,10-anthracenediyldibis(methylene) dima- lonic acid (ABDA, ≥90%), terephthalic acid (TA, 98%), 2,7′- dichlorodihydrofluorescein diacetate (DCFH-DA, ≥97%), phosphate-buffered saline tablet (PBS), sodium acetate (≥99%), molybdenum disulfide (MoS2 99%), hydrochloric acid (HCl, ACS reagent, 37%), glacial acetic acid (pharmaceutical secondary standard), and n-
butyllithium solution were all purchased from Sigma-Aldrich. Ammonia solution (NH₄OH, 30%) was purchased from Daejung chemical. Hydrogen peroxide (H₂O₂, 30%) was purchased from Junsei Chemical Co. Ltd. DiaEasy Dialyzer MWCO 14 kDa was purchased from BioVision. Thioli PEG amine, HCl salt (SH-PEG-NH₂, ≥95%, Mw 2000) was purchased from Jen Kem Technology U.S.A. MTT assay kit (EZ-cyTox) was purchased from Daeil Lab Service co. Ltd., Republic of Korea. The solvents were used as received and without any further purification.

**Synthesis of Gold Nanobipyramid (Au NBPs).** The Au nanobipyramids (Au NBPs) were synthesized by the seed-mediated method. The seed solution was prepared by mixing 0.125 mL (0.01 M) and 0.25 mL of trisodium citrate aqueous solution (0.01 M) in 9.625 mL of water with a freshly prepared, ice-cold 0.15 mL of NaNBH₄ (0.01 M) and 0.25 mL of trisodium citrate aqueous solution (0.01 M) in a plastic tube. The solution combination was mixed by inversion for 10 s, and then the resultant brownish-yellow seed solution was kept at room temperature for 2 h prior to use. The growth solution was prepared in 40 mL of CTAB solution (0.1 M) with the successive addition of 2 mL of HAuCl₄ (0.01 M), 0.4 mL of AgNO₃ (0.01 M), 0.8 mL of HCl (1 M), and 0.32 mL of ascorbic acid (0.1 M). After the colorless solution formed, 0.2 mL of the seed solution was injected into the growth solution mixture, followed by gentle inversion for 10 s, then the solution was left undisturbed overnight at room temperature. The as-synthesized 40 mL of Au NBPs solution was then centrifuged at 11,000 rpm for 20 min. The Ag overgrowth was then carried out by redispersing the precipitate in 20 mL of CTAC solution (0.08 M) and consequently adding 8 mL of AgNO₃ (0.01 M) and 4 mL of ascorbic acid (0.1 M). The bimetallic Au/Ag products were obtained by the reaction of the resultant solution in an oven at 60 °C for 4 h. The bimetallic Au/Ag products were collected by centrifugation at 10,500 rpm for 20 min. Then, the precipitate was again redispersed in 10 mL of CTAB solution (0.1 M) and left uninterrupted overnight at room temperature. The clear supernatant was then collected by centrifugation in 3000 rpm as a supernatant of resultant solution four times at 1 h intervals. The clear supernatant was then taken out carefully and collected by centrifugation (11,000 rpm, 20 min). Finally, the Au NBPs were collected and redispersed in 10 mL of CTAB solution (0.01 M) for further use.

**Synthesis of MoS₂ NSs.** The MoS₂ NSs were synthesized by adopting the Morrison method. Briefly, 0.5 g of MoS₂ crystal was saturated in 0.5 mL of n-butylthiophosphoric acid in hexane (1.6 M) inside a nitrogen glovebox for 2 days. Then, MoS₂ was filtered and repeatedly washed with 80 mL of hexane to remove excess lithium and other organic residues. The MoS₂ sample was removed from the glovebox and then sonicated in water for 1 h. The exfoliated MoS₂ was then collected by centrifugation in 3000 rpm as a supernatant followed by removal of the precipitate. The clear supernatant was then dialyzed using membranes with a molecular weight cutoff (MWCO) of 14 kDa against deionized water for 2 days, and finally the MoS₂ NSs dispersed in water was collected for future use.

**Synthesis of AuNBPs@MoS₂.** Twenty microliters of APTES was added to a 3 mL of aqueous solution of synthesized Au NBPs and stirred for 30 min at room temperature. The product was collected by centrifugation at 8000 rpm for 10 min and dispersed in 3 mL of DI water. Then, the water solution of Au NBPs was added dropwise to 6 mL of aqueous solution of exfoliated MoS₂ (3.0 × 10⁻⁷ M) under vortexing and left undisturbed for 24 h. The AuNBPs@MoS₂ was collected by centrifugation at 8000 rpm for 10 min and washed with water three times and redispersed in water for future use.

**Synthesis of AuNBPs@MoS₂−PEG.** Twenty milligrams of SH-PEG-NH₂ was added to 1 mL of aqueous solution of AuNBPs@MoS₂ (200 µg/mL) under sonication at room temperature and then incubated overnight. The AuNBPs@MoS₂−PEG was collected by centrifugation at 8000 rpm for 10 min and then washed with DI water three times and redispersed in 1 mL of water for future use.

**Peroxidase Activity Measurements.** The peroxidase-like catalytic properties were investigated in 3 mL of acetate buffer solution (0.1 M, pH 4.5) consisting of 0.5 mM TMB, 13 mM H₂O₂, and in absence/presence of AuNBPs@MoS₂ (6.4 µg/mL) at 37 °C. The comparative experiments were conducted in 3 mL of acetate buffer solution (0.1 M, pH 4.5) with (i) TMB and H₂O₂, (ii) TMB and AuNBPs@MoS₂, (iii) AuNBPs@MoS₂ and H₂O₂ in absence of TMB, (iv) TMB and AuNBPs@MoS₂ and H₂O₂, and (v) TMB with AuNBPs@MoS₂ and H₂O₂, respectively. The absorbances at 652 nm were monitored under identical reaction conditions. The kinetic analyses were carried out in 3 mL of acetate buffer solution (0.1 M, pH 4.5) using AuNBPs@MoS₂ (12.5 µg/mL) in the presence of a (i) fixed amount of H₂O₂ (15.2 mM) with differing amounts of TMB solutions (0–1.47 mM) and (ii) fixed amount of TMB (0.5 mM) with differing amounts of H₂O₂ (0–7.46 mM) at 37 °C under 808 nm NIR light illumination. The absorbances at 652 nm were monitored for kinetic analyses. The Michaelis–Menten model (steady state kinetic plot) and Lineweaver–Burk model (double-reciprocal plot) were adopted for evaluation of the kinetic parameters, which is based on eq.1

\[
V = \frac{V_{\text{max}}[S]}{K_s + [S]}
\]

where \(V\) is initial velocity, \(V_{\text{max}}\) is maximum velocity, \([S]\) is substrate concentration, and \(K_s\) is Michaelis constant.

**Detection of ROS.** The generation of ·OH from H₂O₂ catalyzed by AuNBPs@MoS₂ was established by the formation of a fluorescent compound (2-hydroxyterephthalic acid, TAOH) from TA in the presence of ·OH, with a maximum emission peak at 435 nm. In this experiment, 3 mL acetate buffer solution (0.1 M, pH 4.5) containing TA (6 × 10⁻³ M) and AuNBPs@MoS₂ (12.8 µg/mL) was subjected to react with and without 13 mM of H₂O₂. The suspension mixture was stirred for 1 h at room temperature in dark and under 808 nm NIR laser illumination (2 W/cm²) for 1 h, then the photo-luminescence spectra were recorded under the excitation wavelength of 315 nm. The generation of ·OH from H₂O₂ was also evaluated by the use of AuNBPs@MoS₂ under NIR light illumination, in this case, 3 mL acetate buffer solution (0.1 M, pH 4.5) containing TA (6 × 10⁻³ M) and AuNBPs@MoS₂ (12.8 µg/mL) was illuminated under 808 nm NIR laser illumination (2 W/cm²) for 4 h, and the emission spectrum was recorded as mentioned above. The detection of ·O₂⁻ was evaluated using ABDA as a molecular probe. 3 mL acetate buffer solution (0.1 M, pH 4.5) containing ABDA (3 × 10⁻³ M) and AuNBPs@MoS₂ (12.8 µg/mL) was illuminated with 808 nm NIR laser (2 W/cm²) for 4 h, and the UV–vis absorbance spectra in the range of 300–450 nm were recorded at certain time intervals.

**Cell Culture, Cell Viability, and Bioimaging.** The human cervical cancer cells (HeLa cells) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) cell culture medium with fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 mg/mL) at 5% CO₂ at 37 °C. The in vitro cell viability measurements were conducted by the MTT (EZ-cyTox, Daeil Lab Service Co. Ltd., Republic of Korea) assay method. In this case, 1 × 10⁴ number of HeLa cells per well were grown into a 96-well plate in DMEM. After 12 h, different concentrations of AuNBPs@MoS₂−PEG (0–100 µg/mL) in 100 µL DMEM was added to each well for another 12 h incubation. The old medium was removed and wells were carefully washed with 0.1 M PBS. Again, the cells were incubated with MTT (0.5 mg/mL) in DMEM (100 µL/well) for 4 h followed by further removal and incubation with MDM (100 µL/well) containing EZ-cyTox (10 µL) for another 4 h. The absorbance intensity at 450 nm of each well was then measured by using a microplate reader, and the relative cell viability (%) with respect to the control well was calculated with the equation, \(A_{\text{test}}/A_{\text{control}}\) where the average absorbance of the control samples is denoted as \(A_{\text{control}}\) and test sample is denoted as \(A_{\text{test}}\). The combined in vitro therapeutics of AuNBPs@MoS₂−PEG (0–100 µg/mL) for 12 h incubation, the cells were further treated with H₂O₂ (100 µM) and kept for 4 h. The cells were then also illuminated under...
808 nm laser light for 30 min (10 min illumination then 5 min break) (2 W/cm²) and further incubated for 4 h. The absorbance intensity at 450 nm of each well was then measured using a microplate reader and the cell viability was calculated as mentioned above. In contrast, several control experiments were also conducted for comparison, such as, blank control, NIR laser, H₂O₂, H₂O, with laser, MoS₂ with H₂O₂, and AuNBPWS with laser by adopting the above-mentioned technique. In the case of cellular imaging studies, HeLa cells (1 × 10⁵ cells per dish) were implanted in DMEM in plastic bottomed µ-dishes. After growth, the cells were treated with AuNBPWS@MoS₂—PEG (50 µg/mL) and further incubated for 12 h. After washing with PBS and fixing with 4.0% formaldehyde (10 min, room temperature), the imaging slides were prepared through the standard protocol.

In the case of intracellular ROS detection, the HeLa cells were grown in a plastic bottomed µ-dishes (1 × 10⁵ cells per dish) and further incubated with AuNBPWS@MoS₂—PEG (50 µg/mL) for 12 h. The cells were washed with PBS and fresh DMEM containing DCFH-DA (20 µM) was added followed by incubation for another 20 min in dark. The cells were irradiated with 808 laser (2 W/cm²) and the slide was accordingly prepared as mentioned.

**Finite-Difference Time-Domain (FDTD) Simulation Studies.** The optical simulation was conducted using Numerical FDTD Solutions in order to calculate the electric field density. The electromagnetic pulse in the wavelength of 808 nm was used to excite the single Au NBPWS. The periodic boundary conditions for the x-axis and y-axis were used while a perfectly matched layer (PML) condition for the z-axis was used to absorb all the light propagating outward, with a 0.5 nm mesh size. The refractive index of the surrounding background was set to water with the index of 1.33. The sizes of Au NBPs and AuNPWS with laser by adopting the above-mentioned technique. The periodic boundary conditions for the x-axis and y-axis were used while a perfectly matched layer (PML) condition for the z-axis was used to absorb all the light propagating outward, with a 0.5 nm mesh size. The refractive index of the surrounding background was set to water with the index of 1.33. The optical constants of Au and MoS₂ were taken from Johnson and previous literature, respectively. The sizes of Au NBPs and AuNPWS with laser were calculated from the average sizes measured from the TEM image.

**Two-Photon Luminescent Measurements.** The TPL microscopical measurements were performed on a Multiphoton CLSM (LSM510 META NLO, PMT detector, Carl-Zeiss) in which a femtosecond Ti:sapphire laser arrangement was utilized as the excitation source. The excitation wavelength was tunable in the wavelength range from 260 to 2600 nm, and the laser pulse was adjusted at 800 nm. The duration of the laser pulses was 100 fs with the repetition rate of 1.0 MHz. A short-pass filter of 750 nm wavelength was also used before the monochromator beam. Image processing was carried out using the Leica Application Suite Advanced Fluorescence (LAS AF).

**Instruments and Measurements.** TEM images were collected from a JEOL JSM2100F microscope operated at 100 K. Elemental analysis and mapping were accomplished by EDX using a JEOL JEM-301L at accelerating voltage of 200 kV. Scanning electron microscopy (SEM) images were taken using JEOL JSM6700F. Raman spectra were collected using LabHRoevo 800 (HORIBA Jobin Yvon, France). UV—vis absorbance spectra measurements were conducted on a Varian Cary5000 UV—vis—NIR spectrophotometer. Cell viability tests were done using Infinite M200 PRO micro plate reader. PL spectra were collected using a PerkinElmer LS 55 spectrophotometer at room temperature. The 808 nm NIR laser source from Hi-Tech Otoelectronics Co. Ltd. was used to induce photothermal and photodynamic effects.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b15443.

Detailed experimental methods, additional characterization of nanomaterials under different conditions, and detailed peroxidase-like property (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: dhhkim@ewha.ac.kr (D.H.K.).
E-mail: hyukjin@ewha.ac.kr (H.L.).

**ORCID**

Swarup Kumar Maji: 0000-0003-3282-3397
Kyungwha Chung: 0000-0002-6774-4720
Jianfang Wang: 0000-0002-2467-8751
Hyukjin Lee: 0000-0001-9478-8473
Dong Ha Kim: 0000-0003-0444-0479

**Present Address**


**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (2017R1A2A105022387). Dr. Maji is also thankful to the Department of Higher Education, Science and Technology & Biotechnology, Government of West Bengal, India, for granting special study leave from his academic position (Khatra Adibasi Mahavidyalaya, WB, India).

**REFERENCES**


