One-step shell polymerization of inorganic nanoparticles and their applications in SERS/nonlinear optical imaging, drug delivery, and catalysis

Tzu-Ming Liu1*, Jiashing Yu2*, C. Allen Chang3,4,5, Arthur Chiou4,5, Huihua Kenny Chiang4,5,6, Yu-Chun Chuang7, Cheng-Han Wu1, Che-Hao Hsu2, Po-An Chen4 & Chih-Chia Huang4,5,8

1Institute of Biomedical Engineering and Molecular Imaging Center, National Taiwan University, Taipei, 106, Taiwan, 2Department of Chemical Engineering, National Taiwan University, Taipei, 106, Taiwan, 3Department of Biomedical Imaging and Radiological Sciences National Yang-Ming University, Taipei, 112, Taiwan, 4Institute of Biophotonics, National Yang-Ming University, Taipei, 112, Taiwan, 5Biophotonics and Molecular Imaging Research Center (BMIRC), National Yang-Ming University, Taipei, 112, Taiwan, 6Institute of Biomedical Engineering, National Yang-Ming University, Taipei, 112, Taiwan, 7National Synchrotron Radiation Research Center, Hsinchu, 300, Taiwan, 8Department of Applied Chemistry, National University of Kaohsiung, Kaohsiung, 811, Taiwan.

Surface functionalized nanoparticles have found their applications in several fields including biophotonics, nanobiomedicine, biosensing, drug delivery, and catalysis. Quite often, the nanoparticle surfaces must be post-coated with organic or inorganic layers during the synthesis before use. This work reports a generally one-pot synthesis method for the preparation of various inorganic-organic core-shell nanostructures (Au@polymer, Ag@polymer, Cu@polymer, Fe3O4@polymer, and TiO2@polymer), which led to new optical, magnetic, and catalytic applications. This green synthesis involved reacting inorganic precursors and poly(styrene-alt-maleic acid). The polystyrene blocks separated from the external aqueous environment acting as a hydrophobic depot for aromatic drugs and thus illustrated the integration of functional nanoobjects for drug delivery. Among these nanocomposites, the Au@polymer nanoparticles with good biocompatibility exhibited shell-dependent signal enhancement in the surface plasmon resonance shift, nonlinear fluorescence, and surface-enhanced Raman scattering properties. These unique optical properties were used for dual-modality imaging on the delivery of the aromatic photosensitizer for photodynamic therapy to HeLa cells.

Over the past few decades, nanomaterials have been shown to exhibit various optical and magnetic properties and have been integrated with drug carriers for multifunctional applications in biophotonics and nanobiomedicine1–5. Although there are many known strategies to fabricate nanoparticles, the challenge still remains in developing one general method for synthesizing the different types of inorganic nanoparticles while controlling particle size, shape, and composition. The current synthesis reaction keeps complex procedures and undesired toxic reagents at minimum.

Specifically, the shape- and size-control characteristics of surface plasmon resonance (SPR) of Au inorganic nanoparticles (NPs) have been extensively utilized in bio-tracking, chemical sensors, electronic devices, medical therapies, and imaging agents1–15. When the wavelength of incident light coincides with the SPR wavelengths of Au NPs, the efficiency of the local field enhancement effects of the photon-to-thermal conversion1–4, Raman scattering10–12, and nonlinear optical (NLO)13–15 processes can be greatly improved for theranostics. By considering scattering loss and pigment attenuation in photomedicines, recent efforts indicated that the red to NIR wavelength would be a better choice for deep tissue treatment16,17. However, red-NIR-IR excitation wavelengths for these optical applications were still difficult to obtain despite the manipulation of isotropic Au NPs1–15. Various strategies16–27 for the preparation of shape- and size-controlled Au nanomaterials have been developed. These procedures require complex experimental conditions, toxic reagents (i.e., CTAB, organic solvent, and initiator compounds), and multiple synthesis steps to obtain plasmonic Au NPs with the desired SPR band and surface functionality. Packing Au NPs into amphipathic co-polymers might prevent their aggregation under the
previously mentioned physiological conditions by sterically protecting their surfaces and allowing specific functional groups to be exposed to their subsequent conjugations. The intrinsic amphiphatic features of the polymer in the inorganic-polymer hybrid can act as additional absorption sites for drug delivery. Although several reports have successfully employ amphiphatic co-polymers to coat and encapsulate the nanoparticles via a step-by-step strategy through the ligand exchange and micelle formation surface strategies28–33, these methods are still difficult to control synthesis of the nanocrystal core sizes and types accompanying with synchronous self-assembly of co-polymer on the particle surface.

In this study, we first focused on devising a novel green method (water system) which enables us to synthesize size-tunable Au NPs and form Au@polymer nanostructures via a spontaneous redox reaction between a HAuCl4 precursor and the amphiphilic block copolymer poly(styrene-alt-maleic acid) (PSMA) (Figure 1a). Only two reagents were employed and controlling the reaction time enabled the analogue layer-by-layer growth of the PSMA polymer on the Au NPs (Au@PSMA polymer, abbreviated as Au@polymer) through polymer self-assembly and esterification. Intriguingly, the attached polymer layer was degradable by adding esterase or adjusting to basic conditions. We established a red-shifted SPR absorption of the Au@polymer structure by increasing the polymer thickness thereby enhancing the NLO multiphoton fluorescence by excitation at 1250 nm infrared light. The surface of the Au@polymer NPs consisted of hydrophobic polystyrene blocks that could adsorb a large amount of aromatic drugs (i.e., doxorubicin (DOX), melamine blue (MB), and chlorin e6 (C60)) via π–π stacking interactions. We demonstrated that the Au@polymer NP carrier of the MB photosensitizer exhibited shell-dependent, surface-enhanced Raman scattering (SERS) properties. Putting these properties together (Figure 1b), the MB-loaded Au@polymer exhibited SERS and an intrinsic NLO fluorescence, allowing the observation of the distribution of MB molecules delivered to the cell.

PSMA is an inexpensive, commercially available copolymer that has been employed in the synthesis of hydrophobic nanocrystals with carboxylate functional groups in aqueous solutions25–34. Recent studies showed that PSMA micelle composites can serve as nanocarriers due to hydrophobic interactions between the styrene moiety and the anticancer drug35–36. Thus, the use of PSMA polymer is suitable for the development of different nanoobjects. In addition to preparin Au@polymer NPs, we further demonstrated that the polymer-assisted synthesis strategy provides a general, one-pot reaction to prepare other inorganic-organic NPs (Figure 1c), such as metal@polymer and metal oxide@polymer NPs.

### Results

In a typical Au NP synthesis, 10 mL of the PSMA polymer (6 mg/mL) was reacted with 0.25, 0.5, and 1 mL of an HAuCl4 solution at 200°C for 1 h to produce NPs with diameters of ~15 nm, ~19 nm, and ~22 nm, respectively (Figure 2a–c). The particle size was analyzed with transmission electron microscopy (TEM). With the increase of particle size from ~15 nm to ~22 nm, the absorption band of Au NPs shows little red-shift (Figure 2d). The changes of particle diameters and absorption peaks as a function of HAuCl4 concentrations were summarized in Table S1.

Notably, in a high-magnification TEM image (Figure 3a), a thin coating (with low contrast) was observed on the Au NPs (1 h), suggesting that a condensed polymer nanolayer was generated. Au NPs with a diameter of 22 nm were used to study the effect of the reaction time on the polymer deposition because their larger particle size facilitated their observation. Time-dependent TEM images (Figure 3a–c) demonstrated that the polymer shell coating became thicker and the diameter of the Au NPs was almost constant (22–23 nm) yet as the reaction time increased. At 13 h, the structure is similar to stuffed tapioca nanoball. Accordingly, along with an increase in the polymer thickness, the local SPR extinction signal showed the expected bathochromic shift (Figure 3d). It would be ascribed to a change in the proximal environment affecting the dielectric constant of the surrounding media30,31. At the same time, a broader absorption tail extending into the visible region accompanied by a shoulder at 260–300 nm appeared after reaction for 10 h and 13 h. The evolution of the new absorption feature could be associated with the generation of carbonyl groups and/or an increase in the number of conjugated double bonds in the condensed polymer structure37,38. These results are summarized in Table S2 and Figure 3e.

We further employed a Raman spectrometer (Figure 3g) to characterize the Au@polymer nanocomposite structure. Although most of the peaks in Au@polymer NPs are inconsistent with these in free PSMA polymer, the vibration signals appeared for samples at 1–6 h of reaction time indicated the organic materials has been deposited on the Au NPs because only HAuCl4, H2O, and PSMA polymer were reacted. The Raman spectra of the Au@polymer NPs at 13 h showed two broad bands at 1584 cm–1 and 1330 cm–1. This observation has suggested that for the PSMA polymer converting to more condensed carbon structures39 (e.g., amorphous graphite or polycyclic aromatic hydrocarbons). However, the products at 13 h was subjected to analysis by using the synchrotron X-ray powder diffraction pattern (Figure 3f) to prove the formation of the Au@polymer nanos-structure. The sharp peaks of the face-centered-cubic structure of Au

---

**Figure 1** Schemes illustrated the polymer-assisted reduction of the HAuCl4 precursor to prepare Au@polymer NPs a), which exhibited polymer shell-dependent SERS and nonlinear optical enhancements for the observation of microscopic drug delivery in system b). c) The synthesis strategy using the different metal salt precursors to prepare Ag@polymer, Cu@polymer, TiO2@polymer, and Fe3O4@polymer NPs.
(JCPDS No. 89-3697) appeared. Additionally, it exhibited a broadening band at $\sim 14^\circ$ that could be ascribed to the generation of condensed organic composites.

To validate the growth of the PSMA polymer on the Au NPs, we recorded the FT-IR spectra to monitor the changes in the surface structure during the reaction (Figure S1). Note that among these IR peaks, the bands at 1720–1760 cm$^{-1}$ were owing to the overlapping of C=O groups in carboxylic acids, carbonyl moieties, and ester bonds which became to rise in the intensity for the resultant Au@polymer NPs after 10–13 h. To verify the formation of the ester bonds in the inorganic-organic nanocomposites, TEM images observed that the reaction with the esterase caused the dissociation of the polymer shell structures, suggesting that the polymer sheath could be biodegradable (Figure S2). The zeta potentials measured for the as-prepared Au@polymer NPs (at pH = 6.4) at 1 h and 13 h were $-18.6$ mV and $-42.1$ mV, respectively, whose magnitude is increased as the polymer shell getting thicker. Indeed, the presence of electrostatic potential on the particle surface was also verified by attracting inorganic Fe$_3$O$_4$@CTAB and amine-functionalized QDs onto the negatively charged Au@polymer NPs (Figure S3). These results confirmed the numerous carboxylate groups exposed to the outmost surface of Au NPs.

Previous reports indicated that the thermal reaction of a HAuCl$_4$ solution with a carboxylate ligand (e.g., citrate molecule) yielded Au

![Figure 2](image1.png)  TEM images of the Au NPs prepared by the reaction of 0.25-mL a), 0.5-mL b), and 1-mL c) HAuCl$_4$ (5 mM) with 10 mL of PSMA polymer (0.78 mg/mL) at 200°C for 1 h. d) UV-visible spectra for these corresponding Au NPs.

![Figure 3](image2.png)  Time-dependent examinations of the a-c) TEM images from 1 h to 6 h to 13 h and d) UV-visible absorption spectra for the as-prepared Au@polymer NPs (~22 nm in core size). e) Au core size/polymer shell thickness plots f) Synchrotron X-ray powder diffraction pattern of the 13 h sample of the Au@polymer NPs. g) Raman spectra of the 1, 6, and 13 h samples of the Au@polymer NPs, where the * symbol shows the styrene portion of PSMA and the D/G bands refer to the sp$^2$/sp$^3$ hybridized carbon atoms of the graphite structure.
colloids because of the electron donation from the oxidizing COOH groups and the release of CO2 molecules. To study the reducing ability of the PMSA polymer, we reacted different concentrations of the PSMA polymer (60–300 μg/mL) with 1 mL of 5 mM HAuCl4 (Figure S4). A peak at approximately 300 nm appeared during the 1 h thermal reaction with 60–150 mg/mL of PSMA, which is consistent with the UV-visible results. This band originated from the formation of a complex with Au(III) ions. The complete reduction of 5 mM HAuCl4 required at least 300 μg/mL of the PSMA polymer. Additional experiments in which the PSMA polymer was replaced with malic acid and succinic acid as Au reducing agents were performed (Figure S5). Micron-sized Au particles were produced from the reduction of the Au3+ ions. As the same reaction with the PSMA polymer analogues (i.e., poly(4-styrenesulfonic acid-co-maleic acid) sodium salt and poly(maleic anhydride-alt-1-octadecene)), the formation of a colloidal Au solution was detected with TEM and UV-visible spectroscopy (Figure S6). These results indicated that the PSMA carboxylate groups contributed to the Au reduction, while the polymer structure controlled the particle size through steric interactions. We deduced that the continuous polymer self-assembly on the Au NPs due to the increase in the internal hydrophobicity via π-π stacking interaction between polystyrene blocks of PSMA structure.

Then we demonstrated the optical enhancements in the NLO and Raman scattering signals of this new Au-polymer nanostructures. We measured the multiphoton spectra of all single-domain Au@polymer NPs (2–13 h) with a 30 s integration time by exciting them with a 1250 nm femtosecond laser (~100 fs) (Figure 4a). Narrow peaks at 625 nm and 416 nm was assigned to the generation of second harmonic generation (SHG) and third harmonic generation (THG) signals, respectively, because of their triple and double frequency of the fundamental excitation at 1250 nm. The origin of the two-photon excitation and three-photon excitation processes was verified according to the pump-power dependence examinations (Figure S7), which gave the slopes of 2.05 for the SHG signal and 2.9 for the THG signal. It could be the system noise that deviate the results a little bit from ideal dependency. Note that a broad emission band at ~590 nm was raised with the increase of polymer thickness on the Au NPs. It displayed a near squared dependence (~1.7) of yields on the excitation power (Figure S7), suggesting that the broad fluorescence might be coming from the two-photon fluorescence (TPF). The TPF intensity of the Au@polymer NPs at 13 h was approximately 37 times greater than that of the product at 2 h. Since the Au@polymer NPs at 13 h have increased absorption intensity at 600–650 nm, we attribute the enhancement of TPF to the two-photon resonant excitation by 1250 nm laser pulses. As a double check, we added NaOH solution in it (Figure S8). The corresponding TPF intensity of the 13 h sample was remarkably suppressed and accompanied by a blue shift of SPR absorption (Figure S8c). Notably, the TEM image (Figure S8b) revealed the dissociation of the surface-condensed organic coating from the structures, which might be caused by the deprotonation of residual COOH groups and the strong breaking of ester bonds. Although the detailed mechanism involved in the increased TPF intensity of Au@polymer NPs is not clear, it could be the aggregation of Au NPs that resulted in inter-particle coupling, red-shift of SPR band, and enhancement of TPF.

To investigate the Raman scattering performance, the SERS signal of MB molecules on the single-domain Au@polymer NPs was measured (with a 633 nm laser excitation at 17 mW for 1 s) (Figure 4b). The MB photosensitizer was chosen as a model drug for the SERS tag because of its good resonance action by exciting at 633–660 nm. The absorptive amount of MB molecules on the Au@polymer NPs was estimated to be 0.078 mg/mg[Au] for the 1 h sample and 0.12 mg/mg[Au] for the 13 h sample on the basis of the UV-visible measurements. Compared with pure MB, the enhancement factors of the 1623 cm$^{-1}$ peak were approximately $3.7 \times 10^{4}$ (1 h), $1.3 \times 10^{4}$ (2 h), and $2.5 \times 10^{4}$ (6 h). The 13 h sample did not exhibit the characteristic Raman peaks of MB molecules. A similar trend in the shell-dependent Raman signal enhancement was observed (Figure S9) by using a 785 nm NIR laser (80 mW and 5 s). Because thinner polymer coatings resulted in smaller gaps between the spatially isolated Au nanoparticles, we proposed two possible mechanisms for the shell-dependent SERS signals of the Au@polymer NPs substrate. The first involved the SERS signal from the ensemble average of each isolated Au@polymer-MB NP. The MB molecules in the polymer sheath were very close to the outermost edge of each Au core (~2.46 nm or less), which is similar to the isolate particle-based enhanced Raman effect. Second, only the MB molecules were in close proximity to the discrete Au NPs, which would increase the resonance associated with the strong Raman vibration.

In addition to encapsulating positively charged MB dye, we found that the Au@polymer NPs (6 h-sample as example) could successfully encapsulate negatively charged Ce6 and positively charged DOX molecules (Figure 5). The absorption of MB, Ce6, and DOX in the Au@polymer NPs was saturated at 0.10 mg[MB]/mg[Au], 0.34 mg[Ce6]/mg[Au], and 0.73 mg[DOX]/mg[Au], respectively. TEM images revealed that the polymer sheath contrast on the Au NPs increased and became dark gray after the absorption of these aro-

![Figure 4](image-url) a) Nonlinear optical spectra of the as-prepared Au@polymer NPs at different reaction times and b) Raman shift detection of MB-tagged Au@polymer NPs (prepared over 1–13 h) at an excitation of 632.8 nm.
matic molecules (Figure S10). The change in the images strongly suggested that the aromatic drugs were absorbed within the Au@polymer nanocarriers. The peaks of each molecule appeared in the UV-visible and fluorescence spectra of the Au@polymer nanocarriers (Figure 5). The emission intensities of the molecules in the Au@polymer-dye nanocomposites were smaller than those of the individual molecules at the same concentration, suggesting a quenching effect of the Au core interior. Especially, the Ce6-loaded particles had a good fluorescence signal (IVIS® Spectrum), which was suitable for fluorescent imaging in vivo (Figure S11). Our result showed that the payload of the aromatic drugs in the Au@polymer NPs is independent of the charge on the molecule.

However, it is worth to note that the 1 h sample of MB-tagged Au@polymer NPs exhibited strong SERS signals (black curve in Figure 4b). MB molecule is known that 660 nm NIR light is able to induceO2 photogenerated source ~0.5 of quantum yield, and it therefore has potential for application in photodynamic therapy66,67. The delivery of Au@polymer-MB NPs to HeLa cervical cancer cells was therefore examined using the 1 h sample. As shown in Figure 6a, the MTT assay showed that the Au@polymer-MB NPs did not have significant cell toxicity up to 20 μM of MB molecules. Free MB molecules (0–20 μM) were also used for comparison and had toxic effects (dark toxicity) on the cells between 10 and 20 μM. We performed a parallel experiment to monitor the release of MB from the Au@polymer-MB NPs in a PBS solution at 37°C (Figure S12). The UV-visible optical record shows that their release (~8%) into solution at pH 7.4 was limited. The release only obtained under a base condition. In vitro test supports that the π-π interactions between the MB molecules and the polymer sheath of the Au@polymer NPs might inhibit the release of MB via a diffusion pathway. The MB molecules did not leak significantly from the Au@polymer NPs, which would prevent the formation of lesions on the mitochondria and nuclei47.

For the particle-treated cells at 24 h, the accumulation of MB-loaded carriers in the cell body of the HeLa cells was easily confirmed by a SERS microscopy. With a bright-field microscopy (Figure 6b), two individual cells were randomly selected in the rectangle (Figure 6b, red rectangle) for SERS analysis of the signal intensity at 1623 cm⁻¹. The reconstructed SERS imaging with the magenta color contrasts showed that the signal was absent in the nuclei region, in agreement with the keep of MB on the Au@polymer nanocarriers. By using nonlinear optical microscopy, we could directly observe the delivery of the Au@polymer nanocarriers into the cell by monitoring the SHG and THG emissions with high contrast and low background autofluorescence (Figure S13). Nuclei of cells can be clearly identified with negative contrast in the image. Because cells wouldn’t have strong SHG signals or two-photon autofluorescence excited at 1250 nm, these NLO signals certainly came from nanocarriers taken up by cells. The NLO images also indicated that the internalized Au@polymer-MB NPs were located in the cytoplasm of the cells rather than in the nuclei, which is consistent with the SERS imaging results.

Next, we utilized fluorogenic substrate 2’,7’-dichlorodihydrofluorescein diacetate (DCFH-DA), a cell-permeable dye, for detection of singlet oxygen in cells. In this method, colorless DCFH-DA molecules cross cell membranes react with reactive oxygen species to produce green fluorescent DCF. All DCFH-DA stained cells were incubated with samples and illuminate with 660 nm light. Brighter green fluorescence was detected in Au@polymer-MB NPs treatment group more than that by free MB under same drug concentration (Figure 6c and 6d). The level of intracellular ROS presented as an
important indicator for PDT. Our preliminary results showed that the Au@polymer-MB NPs could promote intracellular generation of reactive oxygen species in photodynamic therapy.

Finally, we demonstrated that this polymer-assisted synthesis method is generic allows ones to develop new conceptually designed inorganic-organic nanocomposites (Figure 1), i.e., polymer-coated Ag, Cu, Fe₃O₄, and TiO₂ NPs. In these preparations, the synthesis of these metal and metal oxide NPs required the appropriate reaction modifications through addition of \( \text{N}_2 \text{H}_4 \) as a reductant and HCl in optimizing the experimental parameters to prepare highly uniform and stable NPs (Figure 7a–d). XRD measurements (Figure 7e) revealed that the samples in Figure 7a–d were face-centered cubic (fcc) Ag, fcc Cu, fcc Fe₃O₄, and anatase TiO₂. UV-visible spectra showed the SPR appearance of Ag@polymer and Cu@polymer NPs (Figure 7f). The DLS analysis of various samples in Figure 7a–d was performed and listed in Table S4. Most of the particle hydrodynamic diameters are slightly larger to their corresponded solid forms estimated based on the TEM measurements of Ag@polymer (~262 nm), Cu@polymer (~49 nm), and Fe₃O₄@polymer (~38 nm). The hydrodynamic diameter of TiO₂@polymer NPs is larger than the single particle estimation of TiO₂@polymer NPs (~84 nm) determined by TEM image, indicating the aggregates in the solution. These core-shell NPs all present negatively charged surface over ~20 mV.

The comparative experiments for details and explanation can be found in Supporting Information and Figure S14–S19. As PSMA polymer alone react with AgNO₃, CuCl₂, FeCl₂, and titanium(IV) isoproxide precursors, the reducing ability of PSMA polymer could only react with Ag and form Ag@polymer single-particle. We found that the reduction reaction with \( \text{N}_2 \text{H}_4 \) promoted the formation of plasmonic Cu NPs (Figure S16a) as well as the assistance in the crystallization of Fe₂O₃ (Figure 7e and S17) and TiO₂ (Figure S19) particles under base condition. These reactions are done in acid–free conditions. The hydrolyzed \( \text{N}_2 \text{H}_4 \) known as a reductant donates electrons accompanied with the release of OH- ions into water to precipitate metal oxides via the base-catalyzed reaction. However, the interesting morphologies of multicore@polymer structure were observed for the Ag (~22 nm for each domain) after a reaction including 12 \( \mu \text{L} \) HCl and 100 \( \mu \text{L} \) \( \text{N}_2 \text{H}_4 \), as shown in Figure 7a. The formation of the multicore Ag@polymer NPs resulted in a red-shift of the SPR bands at 448 nm and 545 nm. The coupling of several Ag NPs in a polymer sphere resulted in a red-shift of the SPR bands relative to those of the single Ag NPs (20 nm at 400 nm to 60 nm at 455 nm). Intriguingly, we found that the multicore Ag@polymer NPs could be dissociated by reacting with the esterase to break the ester bond linkage (Figure S2c). As the reaction of CuCl₂, PSMA, and \( \text{N}_2 \text{H}_4 \) added with 18 \( \mu \text{L} \) HCl, a single sharp SPR band appeared for the Cu@polymer NPs at 590 nm (Figure S16d) in contrast to the broad band from the acid-free condition (Figure S16d).

We performed a time-dependent TEM images to monitor the growth process of multi-core Ag@polymer NPs (Figure S15). We observed a homogenous particle growth through a progressive aggregation of Ag embryos at the polymer shell as the reaction time goes. The step-by-step self-assembly of Ag NPs and polymer shell was proposed. In addition, we observed that by adding more acid favored the growth of bigger size polymer particle which resulted in more Ag/AgCl NPs encapsulated in large amount in \( \text{N}_2 \text{H}_4 \)-free (Figure S14b) method. Similarly, several TiO₂ sub-NPs aggregated into rice- and rod-shape (with 18 \( \mu \text{L} \) HCl and 100 \( \mu \text{L} \) \( \text{N}_2 \text{H}_4 \)) within a thick polymer matrix (Figure 7d), while the large particle size coated with thin polymer layer appeared after acid-free synthesis (Figure S19a). The adjustment of acid concentration readily played a vital role to grow bigger polymer size and include large amount of NPs. Note that the interior of Fe₂O₃@polymer (with 100 \( \mu \text{L} \) \( \text{N}_2 \text{H}_4 \))
contained several nanoparticles with ~8 nm for each domain size (Figure 7c). Time-dependent TEM images (Figure S18) gave evidence of Fe₃O₄ core consisted of many small nanocrystals after 2 h of reaction time, indicating that the growth of multi-core Fe₃O₄ NP possibly followed by a clustering interaction through magnetic dipole attractions and forms a single Fe₃O₄ NP. Subsequently, the polymer shell started to grow thicker as the reaction time increase.

The multicore Fe₃O₄@polymer NPs exhibited a superparamagnetic behavior (Figure S20a), most likely due to the small domain size. We demonstrated a typical Fenton reaction toward H₂O₂ decomposition using Fe₃O₄@polymer NPs which gave perox-idase-like catalytic activity than that by using commercial Fe₃O₄ NPs (Figure S20b). The detection in the decomposition of H₂O₂ molecule is accessible by eyes below 5 min. Also, we suggested that the highly dispersed, biocompatible, and magnetic behaviors make these Fe₃O₄ nano-clusters promising for safe use of MR contrast agent. Several TiO₂ NPs together in the polymer sphere might enhance the photodegradation.

**Discussion**

Au@polymer nanocomposites with a tunable Au core size and organic shell thickness were successfully synthesized via a one-pot reaction of HAuCl₄ and PSMA precursors. The formation mechanism was based on the crystallization of the Au NPs followed by the subsequent self-assembly and condensation of the PSMA polymer on the surface after the redox reaction. The coating polymer corona could be degraded by reaction with esterase or under basic conditions. Altering the Au-polymer core-shell structures affected the optical properties of the Au NPs. Thicker polymer coatings resulted in a red-shift in the absorption band and thus resonantly enhanced the two-photon emission at an excitation of 1250 nm. A large SERS signal resulted from a thin coating on the Au@polymer NPs because of the close proximity of the molecule to the Au surface. We demonstrated that amphipathic PSMA-coated Au NPs were very...
stable under physiological conditions and therefore could deliver a high dosage of aromatic drugs and be used as an optically enhanced SERS/NLO contrast in cells. The MB-loaded Au@polymer NPs readily promoted reactive oxygen species generation into cancerous cells to execute efficiency photodynamic therapy. The self-assembly of PSMA in this synthesis strategy could be employed to prepare other inorganic@polymer NPs with appropriate addition of \( \text{N}_2\text{H}_4 \) and HCl reagents to prepare uniform and stable NPs. They showed promise for applying for new drug delivery systems in future with magnetically guided function and as an aromatic absorber following by catalytic decomposition.

Citrated ligand-assisted synthesis is a well-established method to generate Au nanoparticles with spherical shape, but it suffers from less colloidal stability under physiological conditions necessary for good biocompatibility and enhanced drug loading. Compared to the popular Turkевич’s method\(^{4,9,10}\) based on the thermal reduction of aurate ions (AuCl\(_4^–\)) with citrate molecules, the hydrodynamic diameter of as-synthesized Au@polymer NPs were very stable against aggregation in the PBS solution by using the dynamic light scattering measurements, while the citrate-capped Au NPs were not (Figure S22). This may be ascribed to the complete surface protection and strong electrostatic repulsion as Au@silica NPs. In a comparison with most popular Au@silica NPs\(^{41,42}\), both of the soft polymer- and hard silica-shell composites increased the suspension stability of the Au NPs as well as chemically and physically inert surface available. To operate optical changes, we observed that PSMA polymer coatings readily modulate the red-shifting position of the surface plasmon absorbance with an increase in the polymer thickness, which is similar to that by a solid silica coating\(^{43}\). In order to conjugate biomolecules, PSMA polymer-coated inorganic NPs were facile for a directly surface modification treatment to conjugate amine terminal group of the targeting objects, using an EDC/NHS coupling reaction. However, a further modification of the silica coating layer with amino-silane coupled reaction to form Au@silica@amino-silane NPs was necessary to execute the same carbodiimide chemistry with the biological molecule at carboxylate end\(^{44}\).

This is the first report of a facile PSMA-assisted synthesis strategy that incorporates amphiphatic functionality on the surfaces of several inorganic NPs (Figure 1). In the case of Au@polymer NPs synthesis, we demonstrated that the polymer shell thickness is easy controllable at different reaction time. This typical synthesis method showed a direct, one-pot preparation of stable polymer-encapsulated Au, Ag, Cu, Fe\(_3\)O\(_4\), and TiO\(_2\) NPs without resorting to complicated purification. 40 nm Au@polymer NPs were collected by centrifugation and washed with distilled deionized water. To prepare the Au@polymer NPs with core size tunable, 0.25–1.0 mL of an HAuCl\(_4\) solution (5 mM) was added to the PSMA solution (6 mg/mL) before the hydrothermal reaction. Once these reagents were mixed, the resulting solution was transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C for 13 h. These Au@polymer NPs were purified using the aforementioned method of centrifugation and redispersion in water.

Preparation of Au@polymer nanoparticles (NPs). 1 mL of a HAuCl\(_4\) solution (5 mM) was mixed with 10 mL of a PSMA solution (6 mg/mL) under stirring, and then the mixture solution was immediately transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C. After different reaction times (1–13 h), the as-prepared Au@polymer NPs were collected by centrifugation and washed with distilled deionized water. To prepare the Au@polymer NPs with core size tunable, 0.25–1.0 mL of an HAuCl\(_4\) solution (5 mM) was added to the PSMA solution (6 mg/mL) before the hydrothermal reaction. Once these reagents were mixed, the resulting solution was transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C for 13 h. These Au@polymer NPs were purified using the aforementioned method of centrifugation and redispersion in water.

Absorption of Fe\(_3\)O\(_4\) and quantum dot NPs on the Au@polymer NPs. Fe\(_3\)O\(_4@\)CTAB NPs were prepared by the thermal decomposition synthesis following by phase transformation reaction according to the literature\(^{45}\). Qd@655 ITK\(_{\text{PEG}}\) quantum dot (QD-655 nm) was purchased from Invitrogen for use without purification. 40 mL of Fe\(_3\)O\(_4\) (500 ppm) and 40 mL of QD-655 nm (8 μM) were mixed with 200 μL of 30 ppm Au@polymer NPs (13-h sample) for 24 h to prepare Au@polymer-Fe\(_3\)O\(_4\) and Au@polymer-QD NPs, respectively. The NPs were purified using the aforementioned method of centrifugation and redispersion in water before TEM measurements.

Methods

Materials. Poly(styrene-alt-maleic acid) sodium salt, 13 wt. % solution in water (PSMA, \( M_m = 350000 \)) (Sigma-Aldrich); poly(4-dimethylamino-4’-dodecyl co-maleic acid) sodium salt (\( M_m = 20000 \)) (Aldrich), poly(maleic anhydride-alt-1-octadecene) (\( M_m = 30000–50000 \)) (Aldrich), succinic acid (Sigma), maleic acid (99.5%) (Chem Service), quaternary ammonium solution (Life), hydrogen tetrachloroaurate(III) trihydrate (HAuCl\(_4\) 99.9%) (Alfa Aesar), Silver nitrate (AgNO\(_3\)) (Fisher), Copper(II) chloride dihydrate (CuCl\(_2\)·2H\(_2\)O) (Riedel-de Haen), Iron(II) chloride tetrahydrate (FeCl\(_2\)·4H\(_2\)O) (T. Steege), Hydrazine monohydrate (N\(_2\)H\(_4\)·H\(_2\)O 98%) (Alfa Aesar), titanium(IV) isopropoxide (97%) (Sigma-Aldrich), tetraethyl orthosilicate (TEOS, 98%) (Sigma-Aldrich), sodium hydroxide (NaOH, min 99%) (Fullin), melamine blue (MB, high purity) (Alfa Aesar), Chitin 90% (Sigma-Aldrich), eserase (Sigma), 3.3’,5,5’-Tetramethylbenzidine (TMB, ≥99%) (Sigma-Aldrich) were purchased for use without purification.

Preparation of Au@polymer nanoparticles (NPs). 1 mL of a HAuCl\(_4\) solution (5 mM) was mixed with 10 mL of a PSMA solution (6 mg/mL) under stirring, and then the mixture solution was immediately transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C. After different reaction times (1–13 h), the as-prepared Au@polymer NPs were collected by centrifugation and washed with distilled deionized water. To prepare the Au@polymer NPs with core size tunable, 0.25–1.0 mL of an HAuCl\(_4\) solution (5 mM) was added to the PSMA solution (6 mg/mL) before the hydrothermal reaction. Once these reagents were mixed, the resulting solution was transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C for 13 h. These Au@polymer NPs were purified using the aforementioned method of centrifugation and redispersion in water.

Preparation of Fe\(_3\)O\(_4@\)polymer NPs. 0.25–1.0 mL of an HAuCl\(_4\) solution (5 mM) was added to the PSMA solution (6 mg/mL) before the hydrothermal reaction. Once these reagents were mixed, the resulting solution was transferred to a 23 mL Teflon-lined stainless steel autoclave and heated at 200 °C for 13 h. These Fe\(_3\)O\(_4@\)polymer NPs were purified using the aforementioned method of centrifugation and redispersion in water before TEM measurements.

Encapsulation of methylene blue in the Au@polymer NPs. A mixture of 1 mL of an Au@polymer NPs (65 ppmAu) solution and 10 μL of methylene blue solution (50 mg/mL) was added for 18 h for MB absorption on the Au@polymer NPs. After washed with the excess methylene blue was removed by centrifugation. The supernatant was examined with UV-Visible spectroscopy to record the absorbance of the solution and estimate the amount of MB absorbed on the Au@polymer NPs. The MB-loaded Au@polymer NPs were purified by a centrifugation-washing process three times and finally stored in pure H\(_2\)O before use.

Determination of the Raman Enhancement Factor of MB-loaded Au@polymer NPs. For all of the SERS measurement, an 8 μL of MB-loaded Au@polymer NPs solution (~100 ppm) was deposited on the respective Si substrate by using a dip-pen nanoscaler and then slowly dried under a dry nitrogen stream under 30–35%RH. The dry sample resulted in a sphere-shaped area of around 5.3 mm\(^2\). Subsequently, the sample substrates were subjected to Raman spectrometer analysis. The Raman enhancement factor of the Au@polymer-NPs containing substrates was determined using the following expression,

\[
EF = \left( \frac{I_{\text{SERS}}}{I_{\text{free MB}}} \right) \times \left( \frac{N_{\text{MB}} \text{ on the Si wafer}}{N_{\text{MB}} \text{ on the Au@polymer NPs on the Si wafer}} \right)
\]

where \( I_{\text{SERS}} \) and \( I_{\text{free MB}} \) indicate the vibration scattering intensities in the SERS and normal Raman spectra, respectively. \( I_{\text{SERS}} \) and \( I_{\text{free MB}} \) were calculated for the strongest peak at ~1623 cm\(^{-1}\). \( N_{\text{MB}} \) on the Au@polymer NPs on the Si wafer and \( N_{\text{MB}} \) free MB on the Si wafer represent the number of MB molecules on the surface of a single Au@polymer NPs and the free MB molecules deposited on the Si substrate, which excited by laser beam. The diameter of sample coating area is around 0.3 cm. The MB molecules on the Au@polymer NPs was estimated on the basis of the UV-visible measurements (e.g., \( 0.078 \text{ mg/mg} \) for 1 h-sample and 0.12 mg/mg for 13 h-sample) in the EF estimation. If the concentration of the Au@polymer-MB NPs and free MB molecules was homogeneous on the Si wafer.
Cytotoxicity analysis. For the MTT assay, the cell treatments and preparation parameters were based on the previous literature. Hela cells (5000 per well) in the 96-well plate was cultured in Dulbecco’s modification of Eagle’s medium plus 10% fetal bovine serum at 37 °C under 5% of CO2 in air. MB-loaded Au@polymer NPs was dispersed in the fresh medium solution. After 1 day of culture time, the culture medium in each well was then removed, and the seeding cells we separately treated with 0.1 ml of MB, Au@polymer NPs (10 ppm) solution, and free MB molecules at an MB concentration of 10, 1, 0.1, and 0.01 mM. Through 24 h incubation, the culture medium was then removed and replaced with 200 μL of the new culture medium containing 10% FBS reagent. The cells were then incubated for 4 h at 37 °C to allow the formazan dye to form. The culture medium in each well was then removed, and dimethyl sulfoxide (DMSO) (200 μL/well) was added for an additional 10 min of incubation. After the cells were centrifuged, the resulting formazan in each well was transferred to an ELISA plate. The quantification determining cell viability was performed using optical absorbance (540/650 nm) and an ELISA plate reader. In dark toxicity, the culture experiment was carefully worked under dark condition to prevent from the light exposure. Free MB molecule was also following the aforementioned processes to be the control experiment.

SERS microscopic imaging. Before cells for SERS spectral analysis, Hela cells were pre-cultured in an 8 wells of Lab-Tek™ chamber slide (10000 cells per well) for 2 days. After incubation, the original medium was removed and 0.3 ml of MB-loaded Au@polymer NPs (10 ppm) in the medium was added to treat with cells. Through 24 h of incubation time, the supernatant solution was carefully discarded and then the particle-treated cells on the slide are washed with PBS. Subsequently, these cells fixed using 4% paraformaldehyde/PBS for 30 min at 37 °C. The cell-containing chamber slide was then transferred to a Renishaw inVia Raman microscope to obtain cellular images. A 683 nm objective lens with a working distance of 7.0 mm to collect point to point signals with computer-controlled x, y-stage in 4.0 μm that were all set up under the Raman microscope measurement. Each data point was at a 1 s acquisition time. All spectral controls, manipulations, and data analysis were performed using WIRE software from Renishaw.

Nonlinear microscopic Imaging. To evaluate the two-photon fluorescence contrast, the Au@polymer NPs-treated cells were studied. The imaging system is a femtosecond laser-based multiphoton nonlinear optical microscope with sub-micron 3D spatial resolution. The laser wavelength operates at approximately 1250 nm, which falls in the NIR generation window (1200–1300 nm) of most biologic tissues. Compared with the commonly used Ti:sapphire laser (700–1000 nm), this wavelength does not two-photon resonantly excite the sortet band of many endogenous fluorophores in cells and tissues and thus, causes the least on-focus damage. Give its advantages for in vivo imaging in deep tissues, it has been widely applied for studies of cellular and molecular biological imaging in live and clinical use. Because most autofluorescence is suppressed, the optical contrast agents that can efficiently excite at approximately 625 nm have high contrast and benefit deep tissue imaging. The laser beam was XY-scanned by a scanning unit (VF300, Olympus) cascaded with an inverted microscope (IX71, Olympus). The laser beam transmitted a multiphoton dichroic beam splitter (edged at 665 nm) and was focused using a water immersion objective (NA = 1.2, 60×, Olympus). The generated second harmonic generation (SHG) and third harmonic generation (THG) were epi-collected by the same objective. The SHG and THG signals were reflected and then separated by another dichroic beam splitter edged at 490 nm. They were detected separately by two other PMTs. The three signal levels were reconstructed by a computer using software in computer with a 2 Hz frame rate. To image the live cells, a micro--incubator on a microscope was used to create an environment with a temperature of 37 °C in an environment that was 5% CO2/95% air. The temperature of the thermostat (LAUDA Ecoline Steradition RE 204) was set to 50 °C to achieve 37 °C at the distal ends of objective, but the vapor reaching the micro-incubator through the duct maintained the micro-incubator at approximately 37 °C. The gas controller (OKO Lab) continuously supplied 5% CO2, and maintained the outlet absolute pressure at 1 atm. The water immersion objective with 1.2 NA was heated by a dual temperature controller (TC-144, WARNER instrument). This made the temperature at the bottom of the dish that contacted the objective approximately 37 °C.

Intracellular Reactive Oxygen Species Detection. The detection of intracellular reactive oxygen species was measured using fluorogenic substrate 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA). Fifty thousands of Hela cells were stained with 1 ml of DCFH-DA solution (20 μM in PBS) for 30 min in darkness. Thereafter, the cells were incubated with MB-loaded Au@polymer NPs and free MB at the same MB concentration (5 μM) in the DMEM for 2 h. To wash away the free materials, the culture plate was rinsed with PBS buffer two times, and then fresh PBS was added to each well. After 660 nm NIR light illumination (30 mW/cm2) for 4 min, fluorescence microscope (Nikon, Japan) was used to observe the green fluorescence image of cells. We used imageJ software to quantify the fluorescence intensity.

Characterization. Transmission electron microscopy (JEM-2000EX II at 80 KV) was employed to obtain a more accurate of the solid particle size estimate. The dark and gray areas of the core-shell structure in electron micrographs was calculated based on 150 particles and analyzed by using SigmaScan Pro software. 5.0. The absorption and fluorescence spectra of MB-, Ce6-, and DOX-loaded Au@polymer NPs were measured using a UV-Vis spectrophotometer (8452A; Hewlett-Packard Company, Palo Alto, CA) and a FSP 920 fluorescence spectrophotometer (Edinburgh, Instruments, UK), respectively. IR spectra were measured using a Fourier transformation infrared spectrometer (200E; JASCO International Co., Ltd., Tokyo, Japan) by KBr plate. The Au ion concentration was quantified using an inductively coupled plasma atomic emission spectrometer (ICP-AES, JY138 Spectroanalyzer; Horiba Jobin Yvon, Inc., Edison, NJ). The zeta-potential and hydrodynamic diameter of the Au@polymer NPs dispersing in an aqueous solution and a PBS buffer solution were measured using a Zetasizer analyzer (Malvern, UK). Home-built Raman spectra of Au@polymer NPs (at an exposure time of 10 s), MB-loaded Au@polymer NPs (at an exposure time of 1 s), and free MB molecules (at an exposure time of 1 s) were obtained with a 532 × objective lens using Raman microscopes equipped with a 632.8-nm air-cooled He Ne laser (17 mW) as the exciting source. The detailed structure of the imaging system has been described elsewhere. For 785-nm excitation (80 mW), a Renishaw inVia Raman microscope with a 683 × objective lens was applied to analyze MB-loaded Au@polymer NPs under an integrated time of 10 s. Si was utilized as a substrate for Raman examination. Thin film X-ray Diffraction (Bruker AXS GmbH, Karlsruhe, Germany) was utilized to analyze the crystallization of Ag@polymer, Cu@polymer, Fe@polymer, and TiO2@polymer NPs.

The powder X-ray diffraction patterns of Au@polymer nanoparticles were measured at the BL01C2 beamline of the National Synchrotron Radiation Research Center (NSRRC) in Taiwan. The ring energy of NSRRC was operated at 1.5 GeV with a typical current of 360 mA. The wavelength of the incident X-rays was 0.688 Å (18 keV), delivered from the superconducting wavelength-shifting magnet and a Si(111) double-crystal monochromator. The diffraction pattern was recorded with a Mar345 imaging plate detector located 332.10 mm from the sample position and an exposure duration of 5 min. The pixel size of Mar345 was 100 μm. The one-dimensional powder diffraction was converted using the FIT2D program with cake-type integration. The diffraction angles were calibrated based on the Bragg positions of Ag-Benenate and Si powder (NBS640h standards). The powder sample in solution was sealed in a glass capillary with 1.0 mm diameter. To reduce the background signal from solution, a blank experiment with pure solution in capillary was repeated at the same experimental condition. The integrated intensity was obtained after subtracting the background noise.
33. Huang, C. C.
32. Wang, D., Li, Z. C. & Chen L. Templated synthesis of single-walled carbon
24. Chien, Y. H., Huang, C. C., Wang, S. W. & Yeh, C. S. Synthesis of nanoparticles:
23. Metraux, G. S., Jin, R. & Mirkin, C. A. Photoinduced phase separation of gold in
31. Gittins, D. I. & Caruso, F. Tailoring the polyelectrolyte coating of metal
30. Sa´nchez-Iglesias, A.
29. Kim, B. S., Qiu, J. M., Wang, J. P. & Taton, T. A. Magnetomicelles: composite
27. Pang, X., Zhao, L., Han, W., Xin, X. & Lin, Z. A. General and robust strategy for the
25. Kim, J.
19. Enusten, B. V. & Turkevich, J. Coagulation of colloidal gold. J. Am. Chem. Soc. 85,
20. Brust, M., Walker, M., Bethell, D., Schiffrin, D. J. & Whyman, R. J. Synthesis of
34. Huang, C. C.
38. Li, T., Zhou, C. & Jiang, M. UV absorption spectra of polystyrene.
39. Xu, Z., Gao, H. & Guoxin, H. Solution-based synthesis and characterization of a
42. Guan, Z.
41. Vogler, A. & Kunkely, H. Photoreactivity of gold complexes. Coord. Chem. Rev.
34. Huang, C. C.
38. Li, T., Zhou, C. & Jiang, M. UV absorption spectra of polystyrene.
39. Xu, Z., Gao, H. & Guoxin, H. Solution-based synthesis and characterization of a
42. Guan, Z. et al. Band-selective coupling-induced enhancement of two-photon photoluminescence in gold nanocubes and its application as turn on fluorescent probes for cysteine and glutathione. ACS Appl. Mater. Interfaces 4, 5711–5716 (2012).
43. Liu, X.-L. et al. Solution-dispersible Au nanocube dimers with greatly enhanced two-photon luminescence and SERS. Nanoscale 5, 5368–5374 (2013).
44. Li, J. F. et al. Shell-isolated nanonanoparticle-enhanced Raman spectroscopy. Nature 464, 392–395 (2010).
45. Kneipp, K., Kneipp, H., Itzkan, I., Dasari, R. R. & Feld, M. S. Ultrasensitive chemical analysis by Raman spectroscopy. Chem. Rev. 99, 2957–2976 (1999).
46. Tardivo, J. P. et al. Methylene blue in photodynamic therapy: from basic mechanisms to clinical applications. Photodiag. Photodyn. Ther. 2, 175–191 (2005).
47. Lu, Y. et al. Methylene blue-mediated photodynamic therapy induces mitochondria-dependent apoptosis in HeLa cell. J. Cell. Biochem. 105, 1451–1460 (2008).
48. Wioop, H., Lim, M., Munroe, P., Amal R. Understanding the formation of iron oxide nanoparticles with acicular structure from iron(III) chloride and hydrazine monohydrate. Cryst. Growth Des. 11, 1689–1696 (2011).
49. Cheng, D., Zhou, X., Xia, H. & Chan, H. S. O. Novel method for the preparation of polymeric hollow nanospheres containing silver cores with different sizes. Chem. Mater. 17, 3578–3581 (2005).
50. Peng, X. & Ichinose, I. Manganese oxyhydroxide and oxide nanoparticles for high efficiency degradation of organic pollutants. Nanotechnology 22, 015701(1–7) (2011).
51. Schulzendorf, M., Cavelius, C., Born, P., Murray, E. & Kraus, T. Biphasic synthesis of Au@SiO2 core-shell particles with stepwise ligand exchange Langmuir 27, 727–732 (2011).
52. Jana, N., Earhart, C. & Ying. J. Synthesis of water-soluble and functionalized nanoparticles by silica coating. Chem. Mater. 19, 5074–5082 (2007).
53. Chiu, Y. et al. Quantitative and multicomponent analysis of prevalent urinary calculi using Raman spectroscopy. J. Raman Spectrosc. 43, 992–997 (2012).