Plasmonically Coupled Nanoreactors for NIR-Light-Mediated Remote Stimulation of Catalysis in Living Cells

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ABSTRACT: Artificial nanoreactors that can facilitate catalysis in living systems on-demand with the aid of a remotely operable and biocompatible energy source are needed to leverage the chemical diversity and expediency of advanced chemical synthesis in biology and medicine. Here, we designed and synthesized plasmonically integrated nanoreactors (PINERs) with highly tunable structure and NIR-light-induced synergistic function for efficiently promoting unnatural catalytic reactions inside living cells. We devised a synthetic approach toward PINERs by investigating the crucial role of metal-tannin coordination polymer nanoﬁlm—the pH-induced decomplexation-mediated phase-transition process—for growing arrays of Au-nanospheroid-units, constructing a plasmonic corona around the proximal and reactant-accessible silica-compartmentalized catalytic nanospace. Owing to the extensive plasmonic coupling effect, PINERs show strong and tunable optical absorption in the visible to NIR range, ultrabright plasmonic light scattering, controllable thermoplasmonic effect, and remarkable catalysis; and, upon internalization by living cells, PINERs are highly biocompatible and demonstrate dark-ﬁeld microscopy-based bioimaging features. Empowered with the synergy between plasmonic and catalytic effects and reactant/product transport, facilitated by the NIR-irradiation, PINERs can perform intracellular catalytic reactions with dramatically accelerated rates and efﬁciently synthesize chemically activated ﬂuorescence-probes inside living cells.

KEYWORDS: catalytic nanoreactors, plasmonic nanoprobes, bioriented chemistry, bioimaging, coordination polymer

Beyond the capability of natural enzymes, artificial catalysts that can enable and manipulate a variety of abiotic chemical reactions in living systems with high efﬁciency, atomic economy, and chemical versatility are challenging to develop but can ﬁnd a broad range of applications in biochemical manipulations and developing innovative biomedical tools.1 In this context, strategies that can tune the catalytic functions on demand by a remote operation can efﬁciently synthesize bioimaging probes and/or therapeutic molecules selectively within the diseased cells leading to precise and fast diagnosis and efﬁcient therapy with reduced side-effects, but such strategies have been rarely attempted.2 Recently, biorientated catalysts, mainly involving complexes and/or nanoparticles (NPs) of metals, have emerged for in vitro applications such as biomolecular-labeling, intracellular and organelle-targeted molecular delivery, drug synthesis, and pro-drug activation (Table S1).3–9 However, these catalysts-designs take rather long reaction times and realize the products in poor yields, and they do not offer any plug-in to remotely trigger or promote sluggish intracellular catalytic reactions, which is a serious bottleneck for their advancement to broad and extensive applications.

Plasmonic gold (Au)-nanostructures with tunable near-infrared (NIR)-light absorption have been extensively used for therapeutic and diagnostic operations due to their biocompatibility and localized surface plasmon resonance (LSPR)-induced photothermal and photodynamic effects.10–12 In particular, plasmonically coupled branched Au-nanostructures can generate strong electromagnetic ﬁelds due to the optical antenna-effect and have broad and high extinctions in the biomedically useful NIR-region.13–22 But, synthesizing such anisotropic Au-features in well-controlled morphologies and optical properties and their precise integration in hybrid structures is still challenging. Recently introduced “plasmonic-catalytic-hybrid” structures exploiting the LSPR-induced hyperthermia and hot-charge carriers to promote the catalysis upon light exposure are highly challenging to apply for complex biological milieu because of the possible blocking of fully exposed catalytic active-sites by abundant metalphilic...
Figure 1. Plasmonically integrated nanoreactors (PINERs). (a) Synthetic scheme for PINERs and mechanistic illustration of coordination polymer decomplexation-mediated asymmetric Au-growth. (b) Schematic depiction for NIR-light induced intracellular catalysis by PINER.

Figure 2. TEM images of (a) p-SiO$_2$, (b) Au/cat-SiO$_2$, and (c) TA-Fe-Au/cat-SiO$_2$. (d) Bright-field (BF)-STEM, EDS elemental maps (Si green, Au red, and Fe magenta) of TA-Fe-Au/cat-SiO$_2$. (e) TEM images of Au-PINERs with magnified single particle, Au plasmonic nanospheroids (inset) and internal Au-nanocrystals in PINER (inset). (f) BF-STEM, dark-field (DF)-STEM, EDS elemental maps of Au-PINER. (g) SEM image of Au-PINERs. (h) TEM images of Au-PINERs [having plasmonic nanospheroids of 5.8 ± 0.7 nm (top), 7.9 ± 0.9 nm (middle) and 10.1 ± 1.4 nm (bottom) lengths] synthesized from different amounts of HAuCl$_4$ (top, 0.5/mL, middle, 0.8/mL, bottom, 1 mL, 5 mM).
amines- and thiols-rich biomolecules; and additively, the low photothermal conversion efficiencies of plasmonic component would necessitate the use of the probes in large concentrations and excitation laser powers much higher than biologically safe permissible limits.23−37

Herein, we disclose the design and synthesis of plasmonically integrated nanoreactors (PINERs) consisting of reactant-accessible internal catalytic nanospace enclosed by proximal and highly coupled plasmonic Au-nanospheroids, for NIR-light mediated remote stimulation of catalytic reactions inside living cells (Figure 1). For the well-controllable installation of plasmonic nanospheroids in PINER, we introduce a strategy involving metal−organic coordination polymer nanofilm which undergoes phase-transition during the synthesis and guides the asymmetric growth of Au-nanospheroids while protecting the catalytic size of internal nanocrystals (NCs). Owing to the unique “plasmonic-catalytic” hybrid design and synergistic physicochemical properties, PINERs efficiently carried out catalytic reactions inside living cells and synthesized intracellularly activated fluorescence-probes.

■ RESULTS AND DISCUSSION

Synthesis of PINERs. We devised a three-step synthetic method for PINERs starting from porous silica nanospheres (p-SiO₂) (Figure 1a): first, functionalization of catalytic NCs in porous and amine-rich p-SiO₂ leading to M/cat-SiO₂ (M = Au/Pd/Pt), followed by coating with tannic acid-Fe(III) (TA-Fe) coordination polymer nanofilm and finally growth of plasmonic Au-nanospheroids. For this, we started with p-SiO₂ of 78 ± 6 nm diameter and 3.7 ± 0.8 nm average pore size (Figure 2a), which were synthesized by our previously reported method [Experimental Section]. In the next step, to synthesize Au/cat-SiO₂, an aqueous suspension of p-SiO₂ (300 μL, 10 mg/mL) and HAuCl₄ (1 mL, 15 mM) was vortexed for 2 h and the excess HAuCl₄ was washed off with deionized (DI) water (1 mL, 2 times), and a solution of NaBH₄ (200 μL, 100

Figure 3. Characterization of catalytically different PINERs. TEM images of (a) Pd/cat-SiO₂ and (b) Pd-PINERs. (c) DF-STEM and EDS elemental maps (Au red, Si green, Pd yellow) of Pd-PINERs. TEM images of (d) Pt/cat-SiO₂ and (e) Pt-PINERs. (f) DF-STEM and EDS elemental maps (Au red, Si green, Pt purple) of Pt-PINERs. TEM images of (g) h-SiO₂; (h) cat-h-SiO₂ and (i) h-PINERs.
mM) was quickly added to reduce Au(III) to Au(0), including densely populated, uniformly sized (∼2 nm), and homogeneously distributed AuNCs; characterized by transmission electron microscopy (TEM), high resolution TEM (HRTEM), and X-ray photoelectron spectroscopy (XPS) (Figure 2b and Figure S1). In the next step, a modified reported method was used for coating of TA-Fe on Au/cat-SiO_2; an aqueous suspension of Au/cat-SiO_2 was mixed with TA (60 μL, 10 mM) and iron(III) chloride (60 μL, 10 mM) followed by pH adjustment to 10, resulting in an instantaneous thin coating of TA-Fe coordination polymer nanofilm, designated as TA-Fe-Au/cat-SiO_2 (Figure 2c–d). The coordination complexation between gallol-groups of TA and Fe(III) to form TA-Fe could be visualized by turning the color of the solution to reddish-brown and appearance of optical absorption band at 504 nm in UV–vis spectrum, that originated from the facile ligand to metal charge-transfer (Figure S2). Homogeneous coating (∼0.7 nm) of TA-cat-SiO_2 was additionally verified by TEM, EDS elemental mapping, and XPS (Figure 2c–d). Next, the TA-Fe-Au/cat-SiO_2 were dispersed in an aqueous solution of PVP (2%, 600 μL) followed by the successive addition of HAuCl_4 (1.2 mL, 5 mM) and hydroxyl amine (1.2 mL, 50 mM), leading to a reddish-blue colored solution, which gradually turned to dark blue within 15 min at 25 °C due to the presence of Au-PINERS. TEM and HRTEM images of the isolated Au-PINERS showed formation of closely spaced Au-nanospheroids (1.35 ± 2.2 nm length) with well-preserved AuNCs (1.6 ± 0.3 nm) within the internal porous silica core (Figure 2e). The structure of Au-PINERS was further verified by dark-field and bright-field STEM, EDS elemental mapping (Si, Au), and scanning electron microscopy (SEM) (Figure 2f–g). As shown in Figure 2h, the size of the Au-nanospheroids in Au-PINERS was controllable, depending on the amounts of HAuCl_4 used in the reaction—using lesser amounts of HAuCl_4 resulted in smaller Au-nanospheroids. The UV–vis spectrum measurement of the reaction mixture just after adding HAuCl_4 to TA-Fe-Au/cat-SiO_2 revealed the disappearance of the charge-transfer band at 504 nm, possibly due to the HAuCl_4-induced decomplexation between gallol-groups of TA and Fe(III) (Figure S3 in SI). The comparison of Raman spectra of TA-Fe-Au/cat-SiO_2 before and after adding HAuCl_4 showed dramatically diminished characteristic peaks at 522 [ν(Fe-O (C3))], 565 [ν(Fe-O-C(4))]), 1151 [ν(CO)], 1352 [ν(CC)], and 1494 [ν(CC + δ(CHO))] cm⁻¹ due to the absence of coordination bonding between gallol and Fe(III); this observation further validated the HAuCl_4-induced decomplexation process of TA-Fe (Figure S3 in SI). The reaction time-course TEM images during the growth of Au-nanospheroids showed the following: first, the conversion of compact TA-Fe coating to the decomplexed and swelled morphologies of TA-oligomers (within 1 min), followed by hydroxylamine-mediated reductive growth of Au plasmonic nanospheroids on the silica surface; gradually leading to the final Au-PINER structure (Figure S3 in SI). In a control experiment, when we attempted to synthesize Au-PINERS by directly growing Au-nanospheroids on Au/cat-SiO_2 without a TA-Fe-coating step, uncontrolled and inhomogeneous growth of Au was observed and additionally, unwanted conversion of internal AuNCs to larger AuNPs was also noticed (Figure S4 in SI). In another control experiment, p-SiO_2 was directly coated with TA-Fe (without AuNC-functionalization step) and subjected to the formation of Au-PINERS using HAuCl_4 and
hydroxylamine; this resulted in large Au-structures, isolated from p-SiO2 with no presence of Au plasmonic nanospheroids (Figure S4 in SI). This suggests the crucial role of TA-Fe for the synthesis of Au-PINERs—in directing the Au-precursors (Au3+) for hydroxylamine-mediated reductive growth of Au plasmonic nanospheroids selectively, originated on Au-seed attached to the silica outer surface, while simultaneously screening the unwanted overgrowth of internal catalytic AuNCs. And, the prolate shapes of Au plasmonic nano-spheroids possibly evolve from the partial interaction of Au-seed-face with decomplexed TA-oligomers during the fast hydroxylamine-mediated Au-growth.40 Previously, seed-mediated syntheses of smooth and completely closed Au-shell on SiO2-core have been extensively explored, started with the pioneering work by the Halas group; with some examples of plasmonic shells having branched and porous morphologies.14,22,41−43 However, using these methodologies, it is highly challenging to integrate catalytic NCs with plasmonic nano-antennae in a single confined nanoreactor-arrangement.

Further, we diversified our methodology for the synthesis of PINERs having different catalytic metal-NCs (PdNCs and PtNCs) other than AuNCs (designated as Pd-PINER or Pt-PINER) shown in Figure 3. For this, p-SiO2 was treated with Na2PdCl4 (for PdNCs) or Na2PtCl4 (for PtNCs) in the first step (replacing HAuCl4), keeping the rest of the steps the same as before, which resulted in similar TA-Fe-assisted growth-patterns of Au-nanospheroids as was observed in the case of Au-PINER; the resulting Pd-PINERs and Pt-PINERs were characterized by TEM, HRTEM, XPS, and EDS-elemental mapping (Figure 3, Figure S1). Also, upon using hollow aminated silica nanoshell (h-SiO2) (Figure 3), we synthesized h-PINERs devoid of any catalytic NCs and completely hollow interior, enclosed by closely spaced plasmonic Au-nanospheroids.

**Optical and Thermoplasmonic Properties of PINERs.**

As shown in Figure 4a, the UV−vis spectrum of the Au-PINERs showed strong and broad absorption ranging from visible to the NIR range (500 nm to >900 nm), which is a characteristic of extensive multimodal plasmonic coupling.18 The position and the nature of UV−vis absorption depended on the structure of Au-PINERs—gradual red shift and band-broadening were noticed with the increase in size and interspacing of the Au-nanospheroids (Figure 4a and Figure S5). Such optical absorption tunability of Au-PINERs in the NIR range is highly advantageous for biomedical applications because of deep tissue penetration of NIR light, while avoiding the in vivo autofluorescence.14 Figure 4b represents the dark-field images of Au-PINERs having different sizes of nano-spheroids—a gradual and strong bright color transition and scattering signal enhancement were observed when the Au-PINERs with plasmonically coupled closely spaced Au-nanospheroids were formed. As previously established: resonant illumination of plasmonic nanostructures generates hot charge-carriers which upon nonradiative decay produce highly localized hyperthermia, useful in applications such as solar vapor generation, therapeutic delivery, and catalysis at ambient bulk temperatures.23−28 We hypothesized, such highly localized thermoplasmonic effects of Au-PINERs would be advantageous for promoting chemical reactions efficiently at neighboring catalytic sites within nanoreactor’s confined volume; and with this intention, we measured the thermoplasmonic properties of as-synthesized Au-PINERs. As shown...
in Figure 4c, the bulk-temperature of the Au-PINER-solution (20 µg/mL) reached maximum to ~40 °C within 5 min and remained constant further until 10 min of laser irradiation (0.3 W/cm²), whereas a control experiment with only DI water resulted in no measurable change in the temperature. Further, the increase in the bulk solution temperature is well-controllable with respect to the amount of Au-PINERs in the solutions (Figure 4c). Also, the photothermal temperature increase of Au-PINERs solutions (having equal amount of NPs: 70 µg/mL) showed strong dependence on the laser power (Figure 4d). The repetitive photothermal “heating and cooling” experiment showed reproducibility of the photothermal conversions and high photostability of the Au-PINER-structures, which was also confirmed by the TEM image of the isolated Au-PINERs (Figure 4e and Figure S6). The various electromagnetic “hot spots” generated as the result of plasmonic coupling in nanogaps among closely spaced Au-nanospheroids in nanoreactor account for the augmented optical and thermoplasmonic properties; however, in PINERs synthesized from smaller amounts of HAuCl₄, efficient plasmonic coupling effect is missing due to the larger interparticle gaps among smaller size Au-nanospheroids; therefore, enhanced optical and thermoplasmonic properties are not expected in such smaller sized PINERs.¹⁸⁻²² To study the effect of complex biological medium on optical and thermoplasmonic properties of Au-PINERs, Au-PINERs were dispersed in 10% fetal bovine serum (FBS) containing RPMI cell-growth medium (10% FBS in RPMI). UV−vis spectra of Au-PINERs in cell-media exhibited slight broadening of the LSPR-band as compared to the Au-PINERs in DI-water, which could be due to the nonspecific interaction of media-proteins with Au-PINER-surface and change in refractive index of the cell media compared to DI-water (Figure S7). Irradiation of NIR laser (808 nm, 0.3 W/cm²) on Au-PINERs in cell-media resulted in a similar increase in solution temperature as in the case of Au-PINERs in DI-water (Figure S7), signifying the consistency of optical and thermoplasmonic properties of Au-PINERs even in complex biological media.

Catalytic Properties of PINERs in Solution. Further, we tested Au-PINERs for catalytic deprotection reaction of nonfluorescent bis-N,N'-propargyloxycarbonyl rhodamine 110 (Poc-Rho) leading to fluorescent rhodamine 110 (Excitation/ Emission wavelengths = 498/521 nm) as the model.²³ In a typical experiment, laser was irradiated on a mixture of Au-PINERs (0.1 mg/mL) and Poc-Rho solution (5 µL, 20 mM) in an aqueous suspension (2 mL), and the fluorescence intensity of the reaction mixture was measured every 5 min. In a plausible mechanism of the catalytic reaction, Poc-Rho molecules diffuse through the branched and porous plasmonic shell of Au-PINER, interacting with proximal AuNC-catalysts [Au, acting as pi-acid] through terminal alkenes of propargylcarbamate-groups, finally undergoing O-depropargylation reaction.²⁴ The product of the reaction (rhodamine 110), that is highly fluorescent, can be quantitatively monitored by fluorescence measurements (Figure 5a). In Figure 5b, within 5 min of laser-irradiation, reaction mixtures started exhibiting significant fluorescence intensity, which showed fast parabolic increase, reaching to the plateau between 30 and 40 min, at which point the conversion yield of rhodamine 110 was estimated to be >99%. During this reaction, the bulk temperature of the reaction mixture increased from 25 to 38 °C. On the other hand, in the absence of laser irradiation, catalytic conversion of Poc-Rho to rhodamine 110 using Au-PINER showed <10% conversion yield at 25 °C; in a separate experiment, when reaction temperature was externally raised to 38 °C (using oil bath), there was only slight increase in the conversion yield (~16%) (Figure 5c). Further, in a series of control experiments, using Au/cat-SiO₂ (having only catalytic AuNCs), h-PINER (having only plasmonic Au-nanospheroids), and a physical mixture of Au/cat-SiO₂ and h-PINER afforded poor conversion yields (11.8%, 12.4%, and 10.5%, respectively) in the presence of laser, at constant bulk solution temperature (38 °C) (Figure 5d). With the intention of finding thermal conditions (without NIR) to achieve complete conversion of Poc-Rho to rhodamine-110 with Au-PINER, the bulk temperature of the reaction mixture was raised to >60 °C with an oil bath; after 2 h, the reaction afforded maximum ~80% yield, which could not be improved further even at higher temperatures and at extended reaction times. The measured photothermal response of h-PINER was found to be similar to Au-PINER (Figure S8), indicating that Au-nanospheroids are solely responsible for NIR-light-mediated local and bulk solution temperature increases. Also, catalytic activity of cat-SiO₂ was measured under thermal heating conditions at 70 °C, resulting ca. 86% yield of free rhodamine 110, which is slightly higher as compared to Au-PINER (yield ca. 80%), possibly due to the relatively more accessible catalytic sites in cat-SiO₂ (Figure S9). These results corroborate the established synergistic roles of hybrid plasmonic and catalytic components in PINER (Figure 5d).

To test the effect of substrate-size on the reactivity of Au-PINER, two differently sized substrates: N-Poc-benzylamine and N-Poc-octadecylamine were synthesized and reacted with Au-PINER in the presence of NIR-laser (808 nm, 0.3 W/cm²) for 1 h. As shown in Figure S10, bulkier N-Poc-octadecylamine reacted much slower as compared to smaller size N-Poc-benzylamine, resulting in the corresponding free amines in ca. 10% and ca. 95% yields, respectively. Such low reactivity of larger sized N-Poc-octadecylamine could be due to its limited diffusion across the closely located plasmonic Au-nanospheroids. As apparent from the catalytic reactivity of Au-PINER, remaining TA-moieties and PVP stabilizers may have minimal effect on reaction/product transport possibly due to the noncovalent loose and sparse binding of tannic acid and PVP molecules on the surface of Au-PINER. To intentionally block the porosity of the outer plasmonic shell in Au-PINER, methoxypolyethylene glycol thiol (mPEG-SH) (MW 2000) was densely modified on the Au-PINER surface via strong [Au−S] covalent binding. As shown in Figure S10, the depropargylation reaction of N-Poc-benzylamine using mPEG-SH-modified-Au-PINER (PEG-Au-PINER) resulted in a poor yield of benzylamine due to the efficient blockage of molecular transport by densely bound bulky PEG groups, restricting the access toward the internal active sites of PINER.

To test the performance of Au-PINER in a complex biological environment, reaction medium was changed to 10% FBS in RPMI—in the absence of NIR-irradiation at 38 °C, the reaction resulted in only <5% yield of the rhodamine-110 even after 24 h. Such unexpectedly poor yield could possibly be due to the formation of tight serum protein-corona around Au-PINER, which could block Poc-Rho molecules from approaching to the catalytic interiors of Au-PINER.²⁴ The dynamic light scattering (DLS)-based studies of Au-PINERs isolated from the reaction revealed slight increase in the average hydrodynamic diameter (from 115 to 126 nm) and change in zeta potential (from −7.3 ± 1.1 to −16.11 ± 2.3
mV) which indicated the formation of protein-corona on the Au-PINER-surface. However, when the same reaction (after 24 h at 38 °C) was subjected to the NIR-irradiation, the reaction was completed within 30 min (yield ~95%); this indicated the possible NIR-induced perforation of initially formed tight protein corona due to the deattachment or conformational changes in loosely bound proteins, which provide access to the Poc-Rho molecules.45 With the help of a series of control experiments, we rationalized the high performance and superiority of Au-PINERs in carrying out the catalytic reactions in complex biological media. The Au-PINER design is composed of two components: (i) the interior catalytic component, where small size (ca. 2 nm) catalytic nanocrystals are located within the porous silica compartment, and (ii) the surrounding plasmonic component, consisting of arrays of large size (ca. 15 nm) plasmonic Au-nanospheroids with close interparticle nanogaps (ca. 1 nm). In the control experiment with h-PINER where the nanoreactor is devoid of any catalytic nanocrystals and only possesses plasmonic nanospheroids which can only act as NIR-photothermal transducers, the catalytic reaction proceeded sluggishly (Figure 5), suggesting the indispensable role of the proximal catalytic compartment in the nanoreactor design. In another control experiment, using Au/cat-SiO₂, which only consists of catalytic nanocrystals in porous silica nanosphere, was also found to be inefficient in catalyzing the reaction; and additionally mixing h-PINER (photothermal transducer) with cat-SiO₂ could not increase the reaction rate. These control experiments clearly depict that close nanoscale proximity of the plasmonic corona with the catalytic compartment in the Au-PINER structure is crucial for enhancing the reaction rates. In the plasmonic component of Au-PINER, a large number of Au-nanospheroids are spaced with narrow gaps (ca. 1 nm), generating abundant plasmonically coupled “hot-spots” which can efficiently harvest photon-energy to concentrate large electromagnetic fields, generating hot charge carriers and efficient photothermal conversion.20,23−28 Such highly localized photothermal energy generated on the surface of plasmonic Au-nanospheroids is efficiently transferred to the reactions taking place at proximal catalytic nanocrystals within the nanoconfined volume of Au-PINER.33,35 If the plasmonic component is separated from the catalytic component, photothermal energy is quickly dissipated in the bulk aqueous medium and is not effective in promoting the catalytic reactions. Additionally, some of the catalytic nanocrystals in Au-PINER have a conductive interface with the internal ends of Au-nanospheroids; such catalytic sites may

Table 1. Results of Different Catalytic Reactions on a Variety of Substrates Using Different PINERs in Biological Media

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<th>Reaction[ ]; (Nanoreactor)</th>
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<th>Reaction Time</th>
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<td>[Au]-catalyzed O-depropargylation (Au-PINER)</td>
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<td>[Pd]-catalyzed O-deallylation (Pd-PINER)</td>
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involve the transfer the plasmonic hot-charge carriers for efficient catalysis.28 While the bulk temperature of the solution during laser irradiation does increase to ∼38 °C, the local temperature near to the PINERs will likely be much higher. Based on the analysis performed here, it is unclear whether the enhancements observed is due to field enhancements, local temperature increases, or hot electrons/holes. Another crucial role of the plasmonic-component in Au-PINER is as follows: during the catalytic reactions in biological media, a tight protein corona is highly likely to form which restricts the transport of reactants/products across the nanogaps of the outer shell—an NIR-irradiation-induced photothermal effect causes the perforation/removal of protein corona allowing efficient molecular transport and, consequently, enhances the reaction rates. We characterized the presence of protein corona by TEM, after treatment of Au-PINER with cell culture medium (10% FBS in RPMI) for 30 min in the absence and presence of NIR-laser: the TEM image in Figure S11 clearly shows the formation of thin polymeric shell (with a weak contrast), blocking the surface of Au-PINER in the absence of laser, whereas such polymeric shell is absent when NIR-laser (808 nm, 0.3 W/cm²) is irradiated (Figure S11).

For comparing the catalytic efficacy of Au-PINER, Pd-PINER, and Pt-PINER, we tested them for catalytic deprotection reaction of nonfluorescent Poc-Rho leading to the fluorescent rhodamine 110 in the presence of NIR laser (808 nm, 0.3 W/cm²) at constant reaction temperature (38 °C) and found comparable reaction rates in all three cases with product yields 99%, 91%, and 86%, respectively. Further, different PINERs (Au-PINER and Pd-PINER) were successfully applied for a variety of catalytic depropargylation, deallylation, and C–C cross-coupling reactions on a variety of substrates in biological medium (10%FBS in RPMI), affording high yields of the products within short reaction times (Table 1, Figures S12–S20).

Cellular Uptake and Biocompatibility Studies with PINERs. In biological experiments, the used Au-PINER is composed of Au-nanocrystals (ca. 2 nm) modified inside porous silica interior (78 ± 6 nm diameter and 3.7 ± 0.8 nm average pore size) as the catalytic component and surrounded by plasmonic Au-nanospheroids (13.5 ± 2.2 nm length). The overall average diameter of the Au-PINER is ca. 100 nm (from TEM) with a broad UV–vis spectrum ranging from visible to NIR range (500 nm to >900 nm). Among various PINERs, Au-PINER was selected for biological experiments due to the high stability, biocompatibility, and catalytic efficacy of Au-nanocrystals for O-depropargylation reaction. For the studies on live cells, first, we subjected different amounts of Au-PINERs (10, 20, 100, 200, 500 μg/mL) to the cytotoxicity tests, by incubating with two different cell-lines: LNCaP (human prostatic adenocarcinoma) and MDA-MB-231 (metastatic human breast adenocarcinoma) for 48 h (Figure S21). The cytotoxicity results obtained from a commercial Cell Counting Kit (CCK-8, Dojindo lab., Japan) showed high cell viability (88% and 91% for LNCaP and MDA-MB-231, respectively) at Au-PINER concentrations as high as 500 μg/mL (Figure S21), which affirmed high biocompatibility of Au-PINERs. Previously, it has been extensively studied that the laser-induced uncontrolled plasmonic heating can trigger cellular processes such as apoptosis, necrosis, and necroptosis, which can adversely affect the survival of the cells;46 however, such adverse effects can be overcome by controlling the bulk medium temperature lower than the minimum threshold temperature (<43 °C) for hyperthermia-induced cytotoxicity.47–49 Therefore, we carefully controlled the reaction temperature of cellular catalysis experiments by adjusting the amounts of the cell-internalized/associated Au-PINERs and optimizing the laser flux. For this, first we investigated the viabilities of both cells treated with different amounts of Au-PINERs and successive laser irradiation—the amounts of cell-internalized/associated Au-PINERs were controlled by varying the incubation time of Au-PINERs with the cells and washing-off the excess noninternalized/associated Au-PINERs and estimating the % cellular uptake by ICP-OES. ICP-OES estimates the amount of Au-PINERs associated with the cells, which may account for the combination of cell-internalized Au-PINERs as well as membrane-bound PINERs. The treatment of Au-PINERs (100 μg/mL) with both cells (40,000 cells) resulted in the cellular uptake efficiencies 52, 61, 68, and 72% (for LNCaP), and 46, 55, 71, and 75% (for MDA-MB-231) at incubation time 3 h, 6 h, 12 h, and 24 h, respectively. Next, the cells internalized/associated with different amounts of Au-
PINERs were exposed with the laser (808 nm, 0.3 W/cm²) for 30 min and cell viabilities were assessed with MTT assay in each case. As shown in Figure 6a, with incubation time 3 h, the cell viability was negligibly affected after laser irradiation; however, with the further increase in the amounts of Au-PINERs (with increasing incubation time), cell viability gradually dropped (up to ∼80%), suggesting that 3 h incubation time was optimum for in-cell catalysis. Also, as shown in Figure 6b, increasing the laser flux from 0.3 W/cm² to the higher fluxes (0.6, 0.9, 1.2 W/cm²), resulted in loss in cell viabilities (up to ∼70%). These preliminary investigations crucially informed us to use low laser power (0.3 W/cm²) with low amount of Au-PINERs (with cell-incubation time 3 h) for the further catalysis experiments with the cells. Further, the dark-field microscopy images of the Au-PINER-treated cells (incubation time 3 h) clearly exhibited strong light-scattering and bright yellow-orange color, indicative of the internalization/association of plasmonically ultrabright probes into the cells (Figure 6c). In addition, the locations of the PINERs in cytoplasm of the cells were also confirmed by TEM analysis of chemically fixed and sectioned Au-PINER-treated cells (Figure 6d). Noticeably, TEM images show fewer particles inside cells after 3 h of incubation, whereas in the dark field microscopy images the whole area of cells was covered with Au-PINERs, which indicates that part of the Au-PINERs may be bound to the cell-membrane and part of Au-PINERs have been internalized.

**NIR-Light-Induced Intracellular Catalysis by PINERs.** Next, we intended to assess the intracellular catalytic activity of PINERs in LNCaP and MDA-MB-231 cells. In a typical experiment, cells (40,000 cells/well) were incubated with Au-PINERs (0.1 mg/mL) in a 24-well plate for 3 h and washed with PBS three times to remove noninternalized NPs. Further, cells were incubated with fresh media containing Poc-Rho (1 mM) for another 1 h and, subsequently, exposed to the NIR laser (808 nm, 0.3 W/cm²) for 30 min, and laser power and exposure were carefully controlled to maintain the cell medium temperature <40 °C. The intracellular catalytic conversion of Poc-Rho to fluorescent rhodamine 110 was monitored by confocal laser scanning microscopy (CLSM) under the live cell imaging condition which provides the optimum temperature, humidity, and CO₂/O₂ inside the chamber for long-term live

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**Figure 7.** CLSM-based monitoring of catalytic conversion of Poc-Rho to rhodamine 110 using Au-PINER in living cells (LNCaP and MDA-MB-231). (a–b) Bright field optical, CLSM-fluorescence images (FITC filter) and merged image with nucleus-stained fluorescence images in the absence and presence of laser after reaction and (c–d) corresponding flow-cytometry analysis.
cell observation (Figure S22). In Figure 7, CLSM images of the cells after reactions showed bright fluorescence intensity (in FITC-emission filter) due to the generation of fluorescent molecules of rhodamine 110 from nonfluorescent Poc-Rho; whereas in a control experiment, when the same reaction was conducted without NIR-laser, only weak fluorescence signals were detected. Flow cytometric analysis of cells confirmed that the fluorescence in the presence of laser is 2 orders of magnitude higher than that without laser (Figure 7). Furthermore, the fluorescence intensity depended on the time of laser exposure supporting that the intracellular catalytic conversion reaction can be controlled with the time of laser-exposure (Figure 8). The inconsistency between the FACS results presented in Figure 7 and Figure 8 could be due to the different cellular uptakes of Au-PINERs in both different experiments which may have affected the reaction rates. Further, we intended to monitor real-time catalytic activity of PINERs in a single cell (LNCaP and MDA-MB-231) by CLSM-based high-resolution fluorescence imaging; as shown in Figure 8b, as the time progressed, gradual generation of punctate fluorescence started appearing on an entire volume of the cell due to the catalytic conversion of poc-Rho to rhodamine 110, and the fluorescence signals intensified as the reaction progressed (Figure 8b, Movies S1 and S2). During catalysis under NIR-irradiation, all Au-PINERs synthesized free-fluorophores at different locations (inside and on the membrane of the cell); hence, in CLSM images, generation of fluorescence signals was noticed throughout the cell volume. We recorded high-resolution CLSM images with bright field images to monitor the changes in the morphologies of the cells.

Figure 8. Fluorescence-based monitoring of time-dependent controllable catalysis in living cells (LNCaP and MDA-MB-231). (a) Flow cytometry analysis of fluorescence generated as the result of catalytic reaction for different times (5, 10, 20, 30 min), (b) CLSM-based real-time monitoring of fluorescence generation in a single cell as the result of catalytic reaction.
at different times of catalysis experiments and found no observable changes in physical morphology of the cells (Figure S23, Movie S3). In order to further verify any lethal effects of laser irradiation on PINER-containing LNCaP cells, cell viability analysis was conducted after NIR laser (808 nm, 0.3 W/cm²) irradiation for 30 min (bulk medium temperature <40 °C), which resulted in negligible cell death (Figure S24). In order to clearly show that the cells with associated Au-PINERs are fluorescent after NIR laser irradiation and those cells which were not irradiated are not fluorescent, we used the 35 mm clear cover glass-bottom Petri-dish having a well with internal diameter 13 mm with cultured cells and treated with Au-PINER and poc-Rho, and the spot-size of the NIR-laser was adjusted to cover the half part of cell-culture area (Figure S25). After reaction, from the confocal fluorescence microscopic images, we could see that green fluorescence signals were only detected in the area where laser was irradiated (Figure S25).

| CONCLUSIONS |
In summary, we have conceptualized and developed a synthetic approach toward PINERs which is easily customizable to include different reactive catalytic NCs for performing designer organic reactions; and synergistically coupled with plasmonic functionality, catalytic reaction rates can be accelerated and controlled by remotely operated NIR-laser. As compared to the previously known catalytic platforms for bioorthogonal catalysis, which exhibited slow reaction rates (reaction time >24 h) and afforded poor product yields, the present PINER-design not only affects remotely controllable efficient catalysis (reaction time ~30 min, up to >99% yields) but also provides spatiotemporal control to stimulate catalysis in selected cell population. We discovered a crucial role of TA-Fe coordination polymer nanofilm in guiding the growth of Au-nanospheroids while protecting the internal catalytic NCs from size enlargement. Hyperbranched structures of plasmonic shell in PINERs can efficiently harness NIR-light to show plasmon-induced physicochemical properties: tunable LSPR-based optical absorption, well-controllable localized thermo-plasmonic effect and dark-field light scattering; and catalytic NCs, being in close proximity of Au-nanospheroids, demonstrated synergistic and controllable catalytic effect in living cells. Our synthetic approach toward PINERs has wide scope of diversification in modifying different catalytic functionalities for realizing a variety of reactions with the facility of promoting sluggish reaction rates by remotely operable NIR light. In the future, PINERs can be extended to the development of highly selective theranostic platforms switchable in the response to the biorthogonal catalytic reactions.

| EXPERIMENTAL SECTION |

**Synthesis of p-SiO₂ and h-SiO₂**. Aqueous ammonia (28–30%, 200 μL) was injected into a solution of IGEPAL S20 (1.2 mL) and IGEPAL 720 (1.2 mL) in cyclohexane (20 mL) under vigorous magnetic stirring. Then the mixture of TEOS (4.51 M, 100 μL) and TESD (3.56 M, 150 μL) was added two times first, followed by the addition of TMOS (4.62 M, 20 μL) five times into the suspension at every half-hour interval, consecutively. After 24 h, TEOS (4.51 M, 100 μL) was added to the suspension, followed by 48 h stirring at 25 °C. The resulting white colored aminated silica nanoparticles were collected by centrifugation and washed with ethanol (three times) and deionized (DI) water (one time). Further, aqueous solutions of aminated silica nanoparticles of the concentrations 12 and 1 mg/mL were stirred at 70 °C for 1 h to result in p-SiO₂ and h-SiO₂, respectively.

**Synthesis of M-PINERs** (M = Au/Pd/Pt). First, an aqueous suspension of p-SiO₂ (300 μL, 10 mg/mL) was mixed with HAuCl₄ (1 mL, 15 mM) and the reaction mixture was vortexed (1000 rpm) for 2 h at 25 °C. After washing-off excess HAuCl₄ with DI water (1 mL, 2 times), a solution of NaBH₄ (200 μL, 100 mM) was quickly added to form Au/cat-SiO₂. The resulting brown colored Au/cat-SiO₂ were collected by centrifugation and washed with DI water (1 mL, 2 times) and dispersed in DI water for further use. For the synthesis of Pd/cat-SiO₂ and Pt/cat-SiO₂, the same procedure was used replacing HAuCl₄ with Na₂PdCl₄ and Na₂PtCl₄ respectively. In the next step, an aqueous suspension of M/cat-SiO₂ was mixed with tannic acid (60 μL, 10 mM) and iron(III) chloride (60 μL, 10 mM) at pH 10 (adjusted by 1 M NaOH) and the reaction mixture was vortexed vigorously for 30 s at 25 °C, resulting in a thin coating of TA-Fe coordination polymer layer on M/cat-SiO₂ and the resulting particles (designated as TA–Fe-Au/cat-SiO₂) were collected by centrifugation and washed with DI water (1 mL, 2 times) and dispersed in DI water for further use. In the final step, TA-Fe-Au/cat-SiO₂ from the previous step were dispersed in aqueous solution of PVP (2%, 600 μL) followed by the successive addition of HAuCl₄ (1.2 mL, 5 mM) and hydroxyl amine (1.2 mL, 50 mM), leading to a reddish-blue colored solution, which gradually turned to dark blue within 15 min at 25 °C. After reaction, resulting M-PINERs were washed with DI (1 mL, 2 times).

**Dark-Field Microscopy of PINERs**. Cleaned glass slides were treated with 2% (v/v) aqueous solution of 3-aminopropyl-trimethoxysilane (APTS) for 10 s, followed by washing with DI water and drying under nitrogen. Thereafter, 10 μL of a sample (Au-PINER or Au/cat-SiO₂, 0.1 mg/mL) was loaded on the APTS-treated glass slide and sandwiched with a thinner glass slide. The dark-field images were obtained with a Carl Zeiss (DE/Axiovert 200) microscope.

**Photothermal Conversion Measurements**. To measure the photothermal conversion performance of PINERs, the 808 nm laser at different power densities was delivered through a glass vial containing aqueous dispersion of Au-PINERs for different times. And the temperature change of the solution was recorded by the infrared camera.

**Catalytic-Demasking Reaction on Various Substrates Using M-PINERs in Solution**. A solution of M-PINERs (0.1 mg/mL) was mixed with the solution of the substrate (5 μL, 20 mM) in a glass vial (total volume 2 mL), and laser (808 nm, 0.3 W/cm²) was directly exposed to the reaction mixture. % yield of the product was analyzed by measuring fluorescence or HPLC or NMR.

**Standard Calibration Plot for Rhodamine 110 Fluorescence**. The concentration of the product from catalytic reaction on poc-Rho was measured using the fluorescence intensity corresponding to rhodamine 110. For this, a fluorescence standard curve for rhodamine 110 was prepared. The stock solution of rhodamine 110 (0.3 mM) was prepared in water. Successive dilutions were done, and the fluorescence intensities of prepared solutions (S, 10, 15, 20, 25, 30, 35, 40, and 50 μM) were measured (Figure S26).

**Cell Culture and PINERs Treatment**. Prostate carcinoma LNCaP and metastatic human breast adenocarcinoma MDA-MB-231 were suspended in a 96-well plate at a concentration of 10⁶ cells/mL in roswell park memorial institute (RPMI, 100
µL in each well) with 10% fetal bovine serum (FBS) and 1% antibiotic solution ( Gibco, Invitrogen, Karlsruhe, Germany) and cultured at 37 °C and 5% CO₂ for overnight. The culture medium was then replaced with freshly prepared RPMI. For catalysis experiments, cells were seeded at 80% confluence for 24 h before performing the experiments. Different concentrations of PINER solution in 10% serum-containing RPMI were added, and the cells were left in a culture chamber for 3 h.

**Dark-Field Imaging of Live Cells.** The cells were cultured on a 35 mm confocal dish with glass bottom culture dishes (MatTek Corp., USA) and allowed to grow in RPMI medium supplemented with 10% FBS and 1% antibiotics (37 °C, 5% CO₂) overnight. Next, the medium was replaced with fresh culture medium containing PINERs (0.1 mg/mL), and cells were further incubated for 3 h. Thereafter, the glass slide was washed with PBS buffer three times to remove excess PINERs, and images were obtained with a dark-field Carl Zeiss (DE/Axiovert 200) microscope.

**Cell Cross-Section Imaging Using the TEM.** For cell cross-section imaging, Au-PINERs incubated cells were first detached from the well plate. After washing with PBS solution, >5 × 10⁵ cells were fixed for 3 h with modified Karnovsky’s fixative (2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2). After repeated washing with 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C, cells were fixed with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h and then washed with distilled water two times. Fixed cells were En bloc stained at 4 °C overnight using 0.5% uranyl acetate and then dehydrated with a concentrated gradient series of ethanol (30%, 50%, 70%, 80%, 90%, 100%, and 100% ethanol; 10 min for each dehydration step). Infiltrated cells using propylene oxide and Spurr’s resin were polymerized at 70 °C for 24 h. Various sections of the resin block were cut using an ultramicrotome (MT-X, RMC, Tucson, AZ, USA) and stained with 2% uranyl acetate and Reynolds’ lead citrate for 7 min, followed by transferring the section of interest onto a 300 mesh copper TEM grid.

**Cytotoxicity Assay.** The cytotoxicity of various concentrations of PINERs were evaluated using the Cell Counting Kit (CCK-8, Dojindo lab., Japan). 5000 cells per well were cultured in a 96-well plate in 100 µL of RPMI supplemented with 10% FBS at 37 °C and 5% CO₂ atm. After 24 h seeding, cells were incubated with various concentrations (from 0, 10, 20, 100, 200, and 500 µg/mL) of PINERs for 48 h. After incubating for 48 h, the cells were washed thrice with phosphate buffered saline (PBS), and the cell viability was measured by a CCK-8 assay. The metabolic activity of the cells was measured using CCK-8 (a sensitive colorimetric assay for the determination of the number of viable cells after incubating with probes). Ten µL of the CCK-8 solution was directly added to the incubated cells in each well. After 2 h incubation at 37 °C, the amount of formazan dye was measured by a microplate reader (Anthos 2010, Anthos Labtec, Eugendorf, Austria).

**Cellular Uptake Estimation of the PINER.** PINERs were incubated with precultured LNCaP and MCF-7 cells (10⁴ cells/mL) in 24 well plates for 3 h at 37 °C and 5% CO₂ atm. After three times washing with PBS, lysis buffer (100 µL) was added to the cells. The resulting cell lysate was digested overnight using HNO₃ and H₂O₂ (3:1). On the following day, aqua regia (1 mL) was added, and the reaction mixture was left for 3 h. The sample solution was diluted by deionized water to the final solution containing 5% aqua regia. A series of gold standard solutions containing 5% aqua regia were prepared for ICP-OES analysis. The concentrations of internalized gold were measured by ICP-OES and were reported as the cellular uptake (%).

**Intracellular Catalysis Experiments and Flow-Cytometry Analysis.** The cells were cultured in RPMI-medium (1 mL) in 24-well plates (40,000 cells/well) for 24 h at 37 °C (5% CO₂). Thereafter, media of the cells were replaced by fresh media containing Au-PINERs (100 µg/mL in RPMI) for another 3 h. Further, the media of the cells were removed and cells were washed with PBS (3 times) to remove extracellular PINERs. Further, cells (cultured in 1.9 cm² area) were incubated with fresh media containing Poc-Rho (1 mM) for another 1 h and, subsequently, exposed to the NIR laser (808 nm, laser spot size 15 mm) for 30 min, and laser power was carefully controlled to maintain the cell medium temperature <40 °C. After reaction, cells were washed twice with PBS (2 times), harvested with trypsin/EDTA, and dispersed in 2% FCS (in PBS buffer). The intracellular presence of fluorescent compound was analyzed by flow cytometry under 530/30 band-pass emission filters (FITC). The data was acquired by BD LSR Fortessa instrument and analyzed with FlowJo software (TreeStar, Ashland, OR, USA).

**Real-Time Monitoring of Catalysis by Confocal Microscopy.** Cells were plated on an 8-well µ-Slide with 4 × 10⁴ cells/well and grown for 24 h at 37 °C. The medium was removed, and a solution of PINER in RPMI (100 µg/mL) was added and incubated for 3 h at 37 °C. After incubation, cells were washed 3 times with PBS to remove extracellular PINERs. Further, cells were incubated with fresh media containing Poc-Rho (1 mM) for another 1 h and, subsequently, exposed to the NIR laser (808 nm, 0.3 W/cm²) for different times. Confocal reflection microscopy live cell images were obtained using a 100× objective.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.8b04005.

Additional characterization data, detailed experimental procedures, and other information (PDF)

Movie S1 for real-time monitoring of catalytic reactions in LNCaP cells (AVI)

Movie S2 for real-time monitoring of catalytic reactions in MDA-MB-231 cells (AVI)

Movie S3 for monitoring cell morphology during the catalysis experiment (AVI)

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**Notes**

The authors declare no competing financial interest.
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REFERENCES


(28) Yao, G.; Li, J.; Chao, J.; Pei, H.; Liu, H.; Zhao, Y.; Shi, J.; Han, C.; Xian, R. Gold Complex Catalysis Within Live Mice. ACS Nano 2017, 11, 6102–6113.


