Nanozyme-Triggered Cascade Reactions from Cup-Shaped Nanomotors Promote Active Cellular Targeting

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Abstract
Self-propelled nanomotors have shown enormous potential in biomedical applications. Herein, we report on a nanozyme-powered cup-shaped nanomotor for active cellular targeting and synergistic photo-dynamic/thermal therapy under NIR laser irradiation. The nanomotor is constructed by the asymmetric decoration of platinum nanoparticles at the bottom of gold nanocups. Platinum nanoparticles with robust peroxidase-like activity are employed not only as propelling elements for nanomotors but also as continuous O₂ generators to promote photodynamic therapy via catalyzing endogenous H₂O₂ decomposition. Owing to the Janus structure, asymmetric propulsion force is generated to trigger the short-ranged directional diffusion, facilitating broader diffusion areas and more efficient cellular searching and uptake. This cascade strategy combines key capabilities, i.e., endogenous substrate-based self-propulsion, active cellular targeting, and enhanced dual-modal therapy, in one multifunctional nanomotor, which is crucial in advancing self-propelled nanomotors towards eventual therapeutic agents.

MAIN TEXT
1. Introduction
Nanocarriers have recently attracted great attention from diagnostic sensing to drug delivery owing to their unique advantages, for instance, high cargo payload, prolonged systemic circulation and enhanced permeability and retention (EPR) effect [1-4]. Recently, with the merits of operational flexibility, noninvasiveness, low
toxicity, and high spatiotemporal resolution, nanocarrier-based phototherapies have become an innovative strategy to achieve satisfactory therapeutic outcomes [5-12]. However, the therapeutic efficacy may be discounted due to the intrinsic limitations of mono-modal therapy, for instance, the non-selectivity and strong laser intensity of photothermal therapy (PTT), as well as the hypoxic tumor microenvironment and short half-life time and limited diffusion distance of photoactivated singlet oxygen ($^{1}\text{O}_2$) for photodynamic therapy (PDT) [13]. Therefore, it is highly desired to develop multifunctional nanocarriers to achieve maximized synergistic therapy, especially with _in situ_ oxygen production and active delivery abilities.

On the other hand, previous explorations have indicated that passive diffusion would hinder the delivery efficiency of nanocarriers, leading to weak biofilm penetration and nonspecific accumulation in biological environments [14-15]. Active searching and efficient targeting toward lesion location remain a formidable challenge in diagnosis and treatment. Recently, self-propelled micro/nanomotors (MNMs), which convert local or external energies into mechanical motion, have emerged as a novel methodology to drive nanocargoes toward biological targets [16-25]. Particularly, the active cellular searching and internalization capabilities of MNMs can be modulated by regulating their speed and direction [26]. Furthermore, the combination of MNMs with other diagnostic agents and therapeutic strategies would provide a robust approach to develop active and multifunctional nanocarriers for various biomedical applications, such as diagnostic imaging, targeted drug delivery, and minimally invasive surgery [27-34]. Nevertheless, given the promising biomedical applications, several concerns still need to be addressed for the nanomotors, such as the complex actuation systems, unavailable exogenous fuels in biological surrounding or cytotoxic by-products. On this account, nanozyme is emerging as an attractive candidate for driving nanomotors thanks to the attractive features including robust catalytic activity, high stability, and ease of preparation [35-36].

Herein, we report a new design of nanomotor with good biocompatibility and robust self-propulsion capability for enhanced cell penetration, active drug delivery and synergistic dual-modal therapy under single NIR laser irradiation. Specifically, the nanozyme-powered cup-shaped nanomotor (Figure 1) has the following features: (i) Small platinum nanoparticles (PtNPs) _in situ_ grow asymmetrically at the bottom of gold nanocup (GNC). The Janus structure (GNC-Pt) is conducive to generate asymmetric propulsion force to break Brownian motion, resulting in short-ranged directional diffusion, which facilitates broader diffusion areas and efficient recognition toward biological targets. (ii) PtNPs with robust peroxidase (POD)-like activity are employed as propelling elements via catalyzing endogenous H$_2$O$_2$ decomposition. Since H$_2$O$_2$ is overexpressed in most tumor cells [37], such endogenous H$_2$O$_2$-fueled nanomotor demonstrates great potential for active drug delivery in tumor environment. (iii) The GNC-Pt nanomotors serve as _in situ_ O$_2$ generators to improve the restriction of the hypoxia tumor microenvironment in PDT. Moreover, the active diffusion behaviors also facilitate PDT by enabling the accessibility of ICG to $^{3}\text{O}_2$ and expanding the effective diffusion distance for $^{1}\text{O}_2$. Taken together, we demonstrate the excellent performance of nanozyme-powered GNC-Pt nanomotors as active nanocarriers for efficient cellular uptake and enhanced synergistic PDT/PTT, providing insightful perspectives for the fabrications of active and hybrid nanomotors in a variety of biomedical applications.
FIGURE 1: Schematic illustration of the nanozyme-powered GNCs-Pt-ICG/Tf nanomotor for enhanced dual-modal phototherapy upon NIR laser irradiation via a cascaded strategy consisting of the catalytic and photodynamic reactions.

2. Results

2.1. Preparation and Characterization of GNCs-Pt

As illustrated in Figure 2a, GNCs-Pt were fabricated by a facile bottom-up approach. Firstly, GNCs were prepared using octahedral PbS nanoparticles (PbS NPs) as the sacrificial templates (PbS@GNCs). To achieve optimal photothermal effect for PTT upon 808 nm laser irradiation, the SPR band of GNCs was modulated to ~800 nm by precisely adjusting the opening size (Figure S1). Subsequently, to achieve H$_2$O$_2$-fueled self-propulsion, small PtNPs (~2 nm) grew asymmetrically at the bottom of as-prepared PbS@GNCs (PbS@GNCs-Pt) through reducing H$_2$PtCl$_6$ by ascorbic acid. Finally, GNCs-Pt were obtained by selectively dissolving PbS NPs with HCl.

Different from the template-assisted method, the asymmetric growth of gold and PtNPs can be precisely deposited at the high energy sites on PbS NPs and PbS@GNCs, respectively [38]. As a consequence, this method provides favorable conditions for large-scale preparation of GNCs-Pt nanomotors.

The strong extended ultraviolet-visible (UV-vis) absorption band of GNCs-Pt from 600 to 900 nm indicates the efficient photothermal conversion capability under NIR irradiation (Figure 2b). The uniform scattering color and evenly distributed scattering signal in the dark-field microscopic image confirm the excellent monodispersity of GNCs-Pt (Figure 2c and 2h). Additionally, the well-defined cup-shaped structure of GNCs-Pt was revealed by scanning electron microscopy (SEM) and high resolution transmission electron microscopy (TEM) (Figure 2d and 2e). Furthermore, the asymmetric decoration of PtNPs at the bottom of GNCs was confirmed by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) imaging and corresponding elemental mapping (Figure 2f). The average size of GNCs-Pt is 154±11 nm based on a statistical analysis from 150 particles in the SEM images (Figure 2g). The orientation-dependent dipole
patterns in polarization modulation experiments also verify the asymmetric structure of GNCs-Pt (Figure 2i and Figure S2) [39]. Meanwhile, the zeta potential analysis (55.4±0.3 mV) suggests the good stability of GNCs-Pt in water (Figure 2j). Similarly, detailed characterizations of PbS NPs and GNCs were also carried out to confirm the preparation processes of GNCs-Pt (Figure S2 and S3).

FIGURE 2: The preparation and characterization of GNCs-Pt. (a) Schematic representation of the preparation of GNCs-Pt. (b) UV-vis spectra of GNCs (red) and GNCs-Pt (green). (c-e) Dark-field optical microscopic (c), SEM (d), and TEM (e) images of GNCs-Pt. (f) HAADF-STEM images and corresponding elemental maps of GNCs-Pt. (g) Size distribution of GNCs (red, 149±16 nm) and GNCs-Pt (green, 154±11 nm) determined by SEM images (based on 150 particles). Data are represented as mean ± SD. (h) Single-particle scattering spectra of GNCs (red) and GNCs-Pt (green). The gray line is the fitted curve based on Gaussian function. (i) The polarization-dependent scattering response (green circles) from a single GNC-Pt as a function of the angle relative to the optical axis of the polarizer. (j) Zeta potential of hexadecyl trimethyl ammonium bromide (CTAB) stabilized PbS NPs (blue, 48.9±1.1 mV), GNCs (red, 35.8±0.1 mV), and GNCs-Pt (green, 55.4±0.3 mV). Inset: schematic diagrams of corresponding nanomaterials.

2.2. POD-Like Activity of GNCs-Pt
It has been reported that H$_2$O$_2$ is overexpressed and accumulated during the carcinogenesis of normal cells, which can be used to fuel the nanomotors [40]. The POD-like activity of GNCs-Pt was examined with 3,5,3',5'-tetramethylbenzidine (TMB) as the substrate (Figure 3a). As shown in Figure 3b, only GNCs-Pt could efficiently catalyze the oxidation of TMB (oxidized TMB, oxTMB) in the presence of
H₂O₂ (1%, v/v). Negligible oxTMB was observed for TMB treated with GNCs-Pt, GNCs + H₂O₂, GNCs, and H₂O₂, respectively. Additionally, the time-dependent absorbance changes of oxTMB at 652 nm in the above samples are in good agreement with the optical images (Figure 3c). The catalytic rate is dependent on the concentrations of GNCs-Pt and H₂O₂ (Figure 3d and 3e). The steady-state kinetics was also investigated to demonstrate the excellent catalytic activity of GNCs-Pt quantitatively (Figure S4 and Table S1). In addition, GNCs-Pt exhibit high catalytic activity in a broad pH range (pH=3~9) (Figure S5). This merit overcomes the pH limitation of natural enzyme-based nanomotors for biological applications in the acidic tumor microenvironment. These results demonstrate that PtNPs endow GNCs-Pt with excellent POD-like activity, providing an essential prerequisite for self-propulsion by consuming the overexpressed endogenous H₂O₂ in tumor microenvironments.

FIGURE 3: The POD-like activity of GNCs-Pt. (a) Schematic illustration of the POD-like activity of GNCs-Pt with TMB as the substrate. (b) Optical images of the oxTMB produced under different catalytic conditions for 30 min. (I) TMB + H₂O₂, (II) GNCs-Pt + H₂O₂ + TMB, (III) GNCs-Pt + TMB, (IV) GNCs + H₂O₂ + TMB, (V) GNCs + TMB. (c) The TMB oxidation reactions in GNCs or GNCs-Pt solutions with and without H₂O₂ (1%) in the disodium hydrogen phosphate-citric acid buffer (0.1 M, pH 3.0). [GNCs-Pt]=0.27 mg/mL, [GNCs]=0.27 mg/mL, [TMB]=1.0 mM. (d, e) Effects of the concentrations of GNCs-Pt and H₂O₂ on the POD-like activity.

2.3. Active Movement of GNCs-Pt

The active motion of nanomotors has proven to promote cell targeting in biological environments [26, 41]. Disclosing the effect of H₂O₂ on the self-propulsion capability of GNCs-Pt becomes significant and imperative. On this basis, the diffusion behaviors of GNCs-Pt at different H₂O₂ concentrations (0, 1, 2, 3, 5, and 10%) were investigated by single-particle tracking (SPT) (Figure 4a). As shown in Figure 4b, representative trajectories of individual GNCs-Pt in a set of H₂O₂ solutions with different concentrations were recorded by an upright dark-field optical microscope [42]. With the concentration of H₂O₂ increased from 0 to 10%, the average diffusion area of GNCs-Pt greatly expands more than 25 folds from 2.41 to 61.38 μm² during 10 s, and the averaged velocity also increases simultaneously (Figure S6 and S7). In particular, the instantaneous velocity accelerates nearly ten folds (up to 19.5 μm/s, a speed of 127 body lengths per second) due to the robust POD-like activity of GNCs-Pt (Figure 4c). Additionally, the time-ensemble-averaged mean-squared displacement (TE-MSD), and the corresponding effective diffusion coefficient (Dₑ) and anomalous exponent (α) of GNCs-Pt were calculated (Table S2) [43]. Basically, the diffusion
modes can be categorized by $\alpha$: sub-diffusion ($\alpha < 1$), Brownian motion ($\alpha \approx 1$), and super-diffusion ($\alpha > 1$) [44-45]. As depicted in Figure 4d, the curves of TE-MSD versus time interval transform from linear ($\alpha \approx 1$) to parabolic shape ($\alpha = 1.16$) as the concentration of $\text{H}_2\text{O}_2$ increasing from 0 to 10%, indicating the transition from random Brownian motion to certain directional super-diffusion due to the enhanced self-propulsion. Meanwhile, $D_e$ increases rapidly and then reaches a plateau (0.51 $\mu\text{m}^2/\text{s}$) with a gradually expanded distribution (Figure 4e and Figure S8). Interestingly, a second peak of $D_e$ appears at 0.79 $\mu\text{m}^2/\text{s}$ in 10% $\text{H}_2\text{O}_2$ solution, suggesting the enhanced heterogeneity of self-propulsion (Figure 4f). In sharp contrast, there is no discernible differences in $\alpha$ and $D_e$ for GNCs with or without $\text{H}_2\text{O}_2$ (10%) because of the negligible POD-like activity of GNCs (Figure S9). Therefore, GNCs-Pt exhibit $\text{H}_2\text{O}_2$-dependent enhanced motility, resulting in expanded diffusion area for target searching.

FIGURE 4: Enhanced diffusion of GNCs-Pt in long term with different $\text{H}_2\text{O}_2$ concentrations (0, 1, 2, 3, 5, and 10%). (a) Illustration of the self-electrophoresis of a GNC-Pt via catalyzing the decomposition of $\text{H}_2\text{O}_2$. A gradient of electric charge density will be generated across the GNC-Pt as reaction proceeds. Electro-osmotic flow induced by the charge imbalance will then cause GNCs-Pt to move in the direction opposite to that of the fluid flow (red arrow). (b) Trajectories (for 10 s), (c) instantaneous velocity, (d) TE-MSD of GNCs-Pt at different $\text{H}_2\text{O}_2$ concentrations. (e) Dependence of $D_e$ of GNCs-Pt with different $\text{H}_2\text{O}_2$ concentrations. (f) The distributions of $D_e$ of GNCs-Pt with different $\text{H}_2\text{O}_2$ concentrations. The dashed lines are the fitted curve based on Gaussian function.

Interestingly, it is noteworthy to mention that TE-MSD of all individual trajectories exhibits some disparate behaviors from the time-averaged MSD (TAMSD), indicating the time-dependent heterogeneous of the self-propulsion behaviors within a single trajectory (Figure S10) [46]. The trajectories with or without $\text{H}_2\text{O}_2$ (10%) are illustrated via color-coded speed (Figure 5a). The nanomotor moves in a manner similar to waiting-hopping as it was confined by the crowded medium, which is essential for efficient searching (more examples are shown in Figure S11). Although the SPT technique has been used to reveal the heterogeneous behaviors between individuals, the precise characteristics and dynamics of individual
nanoparticle at different stages are still ignored, such as diffusion mode alternation. This can be concealed by ensemble-averaged measurement over a long period of time. To address this limitation and reveal the directionality of the nanomotors, we further investigated individual trajectories by a moving time-window method.

A typical trajectory of GNCs-Pt in 10% H$_2$O$_2$ was divided into 10 pieces sequentially by a moving time-window of 1.0 s, and TA-MSD was also calculated in each window (Figure S12-14). As shown in Figure 5b, the statistical results according to a series of trajectories at different H$_2$O$_2$ concentrations were obtained. It turns out that although the dominant diffusion mode is H$_2$O$_2$-dependent, GNCs-Pt normally undergo three diffusion modes alternately rather than one or two of them. The higher H$_2$O$_2$ concentration, the greater probability of super-diffusion with higher $D_e$ is observed, providing promising potential for active transport in tumor environment by utilizing overexpressed endogenous H$_2$O$_2$ (Figure 5c).

**FIGURE 5:** The temporal heterogeneity of diffusion behaviors of GNCs-Pt in the absence or presence of H$_2$O$_2$ (10%). (a) Trajectories of GNCs-Pt with the color-coded speed during 10 s periods. The color bar from purple to deep red represents the speed from 0 to 20 μm/s. (b) The distributions of diffusion modes including sub-diffusion, Brownian motion (BW), and super-diffusion. (c) The distributions of $D_e$ of GNCs-Pt by a moving time-window method (for 1.0 s period).

The searching efficiency of nanoparticles is determined by their diffusion behaviors, such as Brownian motion, Lévy walk, and Lévy flights [47]. To further understand the influence of H$_2$O$_2$ on the directionality in the diffusion process, the distribution of azimuthal angle displacement ($\phi$) of GNCs-Pt in solution with different H$_2$O$_2$ concentrations was examined [48]. For comparison, we took the same trajectories in Figure S12 as examples. Interestingly, GNCs-Pt undergo more directional diffusion during each time window in 10% H$_2$O$_2$, which is averaged in the whole trajectory analysis (Figure S15). In sharp contrast, the isotropic random Brownian motion of GNCs-Pt without H$_2$O$_2$ is observed via the moving-windows analysis or whole trajectory analysis (Figure S16). Taken together, these results illustrate that GNCs-Pt possess H$_2$O$_2$-dependent accelerated and short-ranged directional diffusion, which can greatly expand the searching area and facilitate cellular recognition and membrane penetration performance [49]. Meanwhile, the generated O$_2$ can modulate the hypoxia tumor microenvironment, which holds great potential for enhanced PDT.

2.4. Tf and ICG Loading and Characterization

Inspired by the enhanced self-propulsion movability and O$_2$ production ability, we conceive that GNCs-Pt can serve as active nanocarriers for synergistic PDT/PTT under NIR laser irradiation. Briefly, ICG with excitation wavelength at ~800 nm was loaded on GNCs-Pt (GNCs-Pt-ICG) via electrostatic adsorption for efficient
photodynamic reaction by taking full use of the produced O$_2$. Although the enhanced self-propelled movability could increase the searching efficiency and drive the nanomotor toward biological targets, it is still difficult to bind on the cell membrane and be internalized by cancer cells due to the lack of specific recognition and binding capability. Because Tf receptor (TfR) is overexpressed on most of cancer cell membranes, we decorate Tf on the nanomotors (GNCs-Pt-ICG/Tf) to improve the recognition ability toward cancer cells. Furthermore, the nanomotors were modified with methoxy polyethylene glycol thiol (mPEG-SH) to improve the colloidal stability and reduce the cytotoxicity in biological applications. As a control, GNCs were also modified with the same methods (GNCs-ICG/Tf). The successful decorations of ICG and Tf have been proved by UV-vis absorption spectra, zeta potential analysis, and Fourier transform infrared (FT-IR) spectroscopy (Figure S17, Table S3). In addition, the loading capacity is 25.97 mg ICG (33.51 μmol) for 1 g GNCs-Pt (Figure S18).

2.5. $^1$O$_2$ Generation

Abnormal metabolism of cancer cells leads to the accumulation of H$_2$O$_2$ in tumor environment. Because of the POD-like activity, GNCs-Pt-ICG/Tf can serve as O$_2$ generators to promote the PDT effect by consuming overexpressed H$_2$O$_2$. Figure 6a illustrates the cascade concept of the catalytic decomposition of H$_2$O$_2$ and the enhanced photodynamic reaction of ICG. To verify this conceive, $^1$O$_2$ production ability of GNCs-Pt-ICG/Tf was studied under 808 nm laser irradiation (2 W/cm$^2$) by using singlet oxygen sensor green (SOSG) as the indicator (Figure 6b). The slight $^1$O$_2$ generation by GNCs-Pt-ICG/Tf without H$_2$O$_2$ can be attributed to the photodynamic reaction of the loaded ICG with residual O$_2$ in PBS (Figure 6c). However, as a control, $^1$O$_2$ generated from equivalent free ICG (9.05 μM) was much lower than that of GNCs-Pt-ICG/Tf with the same laser irradiation, which can be ascribed to the intrinsic poor solubility and stability of free ICG (Figure 6d and Figure S19). As shown in Figure 6d, only in the presence of both laser (808 nm, 2 W/cm$^2$) and H$_2$O$_2$ (1%), the fluorescence intensity of SOSG sharply increased over 2 times than that of GNCs-ICG/Tf, indicating the generated O$_2$ from the first stage of cascade reaction could accelerate $^1$O$_2$ generation. This holds promising potentials in enhanced PDT due to the following three points: 1) the consecutive generation of O$_2$ addresses the inherent limitation of hypoxia tumor environment; 2) the challenge of poor solubility and biological stability of ICG is greatly improved by the nanomotor; 3) the active diffusion behaviors enable the accessibility of ICG to $^3$O$_2$ and expand the effective diffusion distance for $^1$O$_2$.

2.6. Photothermal Performance

Because of the strong absorption in the NIR region, GNCs-Pt-ICG/Tf would possess good photothermal conversion efficiency for potential tumor treatment. As shown in Figure 6e, GNCs-Pt-ICG/Tf and GNCs-ICG/Tf with concentration of 137 μg/mL were irradiated with 808 nm laser (2 W/cm$^2$) for 10 min. The temperature of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf solution increased from 30 °C to 74.4 °C and 73.3 °C, respectively. However, under the same conditions, the temperature of phosphate buffer saline (PBS) solution and deionized water only ascended to 42.0 °C and 36.5 °C, respectively. The photothermal conversion efficiencies (η) of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf are calculated to be 44.31% and 41.09%, respectively, which are comparable to that of commonly used nanomaterials for PTT such as gold nanorods (39.2%) [50], Cu$_3$BiS$_3$ nanorods (40.7%) [51], and Pt-CuS nanoparticles (34.5%)
These results indicate that the deposition of PtNPs has negligible influence on the photothermal performance of GNCs. In addition, there was negligible temperature deterioration in these two samples during the five “on/off” irradiation cycles, indicating the excellent photothermal stability and reproducibility of GNCs (Figure 6g). All these results demonstrate that GNCs-Pt-ICG/Tf would be a promising candidate for photothermal applications.

FIGURE 6: The ROS generation and photothermal properties of GNCs-Pt-ICG/Tf. (a) Schematic illustration of the mechanism of GNCs-Pt-ICG/Tf for synergistic PDT/PTT upon NIR laser irradiation via a cascade reaction. (b-d) The ROS generation ability of GNCs-Pt-ICG/Tf with SOSG as an indicator. (b) GNCs-Pt-ICG/Tf in the presence of H₂O₂ (1%) with and without 808 nm laser. (c) GNCs-Pt-ICG/Tf in the absence of H₂O₂ with and without 808 nm laser. (d) GNCs-Pt-ICG/Tf and free ICG in different conditions. [nanomaterials] = 270 μg/mL; [ICG] = 9.05 μM; 808 nm laser: 2 W/cm². (e-g) Photothermal properties of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf. (e) Temperature evaluation of GNCs-Pt-ICG/Tf, GNCs-ICG/Tf, PBS and deionized water with 808 nm laser irradiation for different times. (f) A plot of −lnθ versus time obtained from the cooling period for 15 min. (g) The photostability of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf in PBS with 808 nm laser on and off for five cycles. [nanomaterials] = 137 μg/mL; PBS: 10 mM, pH=7.4; 808 nm laser: 2 W/cm².

2.7. Biological Stability and Cytotoxicity
Good biological stability and biocompatibility are two essential factors to evaluate the performance of nanoparticles in biological applications. The stability of GNCs-Pt-ICG/Tf was explored with dark-field optical microscopy at the single-particle level. GNCs-Pt-ICG/Tf display good monodispersity in H2O, PBS, and Dulbecco’s Modified Eagle Medium (DMEM) (Figure S21). In contrast, obvious aggregations from GNCs-Pt (stabilized by CTAB) in PBS and DMEM were observed (Figure S22). Subsequently, the cytotoxicity of GNCs-Pt-ICG/Tf was evaluated using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Figure 7a). HepG2 cells were cultured with different concentrations of GNCs-Pt-ICG/Tf (0, 1, 5, 10, 25, and 50 μg/mL) in dark for 24 h. The survival rate of HepG2 cells is higher than 85% even at a high concentration of 50 μg/mL. In sharp contrast, distinct cytotoxicity (11.5% cell viability) from GNCs-Pt was observed at a low concentration of 5.0 μg/mL (Figure S23). These results show that modification of mPEG-SH is necessary to improve the biostability and biocompatibility of nanomotors (Figure S24 and S25).
FIGURE 7: Cytotoxicity and antitumor efficacy of GNCs-Pt-ICG/Tf in vitro. (a) HepG2 cell viability incubated with GNCs-Pt-ICG/Tf of various concentrations (0, 1, 5, 10, 25, and 50 μg/mL) for 24 h. (b) The effects of PtNPs and Tf/ICG modification on the cellular uptake efficiency of GNCs-Pt-ICG/Tf. Inset: schematic diagrams of the corresponding nanomaterials. (c) The cellular uptake capability of (i) GNCs-Pt-ICG/Tf, (ii) GNCs-Pt-ICG and (iii) GNCs-ICG/Tf, (iv) GNCs-ICG for HepG2 cells, (v) GNCs-Pt-ICG/Tf and (vi) GNCs-Pt-ICG for NCTC1469 cells respectively. CLSM images (d) and corresponding cell mortality (e) of HepG2 cells treated with (I) culture medium, (II) GNCs-Pt-ICG/Tf + laser, (III) GNCs-Pt-ICG/Tf (in dark), (IV) GNCs-Pt-Tf + laser, (V) free ICG + laser, and (VI) GNCs-ICG/Tf + laser, respectively. Scale bar: 50 μm. (f) CLSM images of HepG2 cells co-cultured with GNCs-Pt-ICG/Tf at the edge of laser irradiation. Scale bar: 100 μm. [nanomaterials]=50 μg/mL; [ICG]=1.68 μM; Laser: 808 nm, 2 W/cm².

2.8. Cell Targeting and Uptake
As efficient cellular targeting and internalization are important for cancer treatment, the self-propelled diffusion of GNCs-Pt-ICG/Tf on HepG2 cell membrane before internalization was recorded and analyzed by SPT. Much broader diffusion area and faster instantaneous velocity and $D_e$ from GNCs-Pt-ICG/Tf ($v_{max} =8.31 \mu m/s$, $D_e =0.031 \mu m^2/s$) were observed than those of GNCs-ICG/Tf on living cell membrane ($v_{max} =2.95 \mu m/s$, $D_e =0.006 \mu m^2/s$) (Figure S26). These results clearly demonstrate that the self-propulsion can noticeably increase the active diffusion of nanomotors in biological media.

In addition, cellular uptake of GNCs-Pt-ICG/Tf was investigated in HepG2 cells with dark-field microscopy (Figure 7b). The amount of GNCs-Pt-ICG/Tf within the cells can be counted individually under low incubation dosage. According to the single-particle counting results, the number of GNCs-Pt-ICG/Tf in HepG2 cells is higher than that of GNCs-ICG (over 80 folds) or GNCs-ICG/Tf (over 10 folds), respectively, indicating that the self-propelled diffusion of nanomotors could significantly promote the cellular recognition and uptake (Figure 7c). As a control, the number of internalized GNCs-Pt-ICG (without Tf) is far fewer than that of GNCs-Pt-ICG/Tf, which is because the active diffusion only enhances the accessibility to the cell membrane, but not the uptake efficacy. In other words, the functionalization of nanomotors with Tf is crucial for cellular recognition and uptake, while the accelerated movement of nanomotor could improve these processes. In addition, NCTC1469 cells (a mouse fibroblasts cell line) with negligible surface expression of TfR were selected as another control to explore the specific recognition ability of GNCs-Pt-ICG/Tf toward cancerous cells (Figure S27). As expected, the number of GNCs-Pt-ICG/Tf in the NCTC1469 cells is much less than in the HepG2 cells.

2.9. Enhanced Dual-modal Phototherapy by NIR Irradiation
Encouraged by the performances of GNCs-Pt-ICG/Tf in active cellular recognition and uptake, as well as photothermal and photodynamic capability, we further investigated the synergetic PDT/PTT efficacy with 808 nm laser irradiation (Figure 7d and 6e). The therapeutic efficacy was examined on the basis of cell viability outcomes by treating HepG2 cells with GNCs-Pt-ICG/Tf + laser, GNCs-Pt-ICG/Tf (in dark), GNCs-Pt-Tf + laser, ICG + laser, and GNCs-ICG/Tf + laser, respectively. As expected, negligible toxicity was detected when the cells were incubated with GNCs-Pt-ICG/Tf without 808 nm laser irradiation, which is consistent
with the results in MTT assay. In contrast, \(^{1}\text{O}_2\) generation capability of GNCs-Pt-ICG/Tf is activated upon 808 nm laser irradiation (2.0 W/cm\(^2\), 5 min), resulting in apoptosis for more than 96.4% of cells. Moreover, the mortality of the cells incubated with GNCs-Pt-ICG/Tf is distinctly higher than the total mortality with GNCs-Pt-Tf (47.4%) and free ICG (8.5%) groups, suggesting that the cascade strategy can greatly enhance the PDT/PTT compared to that of single therapeutic model. Furthermore, half of the cells (treated with GNCs-Pt-ICG/Tf) within an observation window were illuminated with laser and then the images of cells in the laser edge area were also captured (Figure 7f). There is a clear dividing line, indicating the negligible cytotoxicity of GNCs-Pt-ICG/Tf in dark, while the potent therapeutic effect upon 808 nm laser irradiation.

3. Discussion

In summary, we introduce a nanozyme-powered cup-shaped nanomotor (GNCs-Pt-ICG/Tf) via a facile bottom-up method for enhanced synergistic PDT/PTT upon NIR laser irradiation. The asymmetric growth of PtNPs endowed the nanomotor with accelerated (up to 19.5 \(\mu\text{m/s}\)) and short-ranged directional self-propelled diffusion by catalyzing the decomposition of overexpressed endogenous \(\text{H}_2\text{O}_2\). This feature boosts the diffusion area and recognition efficiency. As a result, the cellular uptake efficiency of GNCs-Pt-ICG/Tf by HepG2 cells is around 10 folds higher than that of GNCs-ICG/Tf. Meanwhile, the generated \(\text{O}_2\) promotes the photodynamic reaction of ICG, which enhances the PDT effect by overcoming the inherent limitation of hypoxia in tumor environments. Furthermore, the efficient photothermal conversion of GNCs-Pt-ICG/Tf enables the synergistic phototherapy, resulting in the distinctly higher cell mortality after treatment (96.4%). Such a cascade strategy consisting of nanozyme reaction and photodynamic reaction can be generalized to other types of nanomaterials (e.g., Au, Fe\(_3\)O\(_4\), and Cu\(_x\)O nanoparticles) or reactions (e.g., Fenton-like reaction). The efficient cellular targeting and boosted dual-modal phototherapy achieved by the nanozyme-powered nanomotor provides a new strategy of designing multifunctional nanocarriers in a controlled and active manner.

4. Materials and Methods
4.1. Materials

Unless otherwise noted, the starting reagents were purchased from commercial sources and used directly without further purification. Lead acetate (Pb(AC)\(_2\), 99.5%), acetic acid (HAc), thioacetamide (TAA), cetyltrimethylammonium bromide (CTAB), chloroaucric acid (HAuCl\(_4\)•3H\(_2\)O, \(\geq 99.9\%\)), hydrochloric acid (HCl, 36%~38%), chloroplatinic acid hexahydrate (H\(_2\)PtCl\(_6\)•6H\(_2\)O, \(\geq 99.9\%\)), ascorbic acid (AA, \(\geq 99.0\%\)), methoxy polyethylene glycol thiol (mPEG-SH, MW \(\approx 6000\)), N-hydroxysuccinimide polyethylene glycol thiol (NHS-PEG-SH, MW \(\approx 6000\)), and polyetherimide (PEI, MW\(\approx 6000\)) were purchased from Aladdin Reagent Co. Ltd (Shanghai, China).

Hydrogen peroxide (H\(_2\)O\(_2\), 30 wt.% in H\(_2\)O), indocyanine green (ICG) and human transferrin (Tf, MW\(\approx 79\) kD, 98%) were bought from Sigma-Aldrich (St. Louis, Mo, USA).

Bis(p-sulfonatophenyl)phenylphosphate dihydrate dipotassium salt (BSPP, 97%), tri(hydroxymethyl) amino methane hydrochloride (Tris-HCl, 99%) were purchased from J&K Scientific (Beijing, China).
Dulbecco’s Modified Eagle Medium (DMEM), penicillin/streptomycin (PS, 100×), fetal bovine serum (FBS), and trypsin were brought from Gibco (Carlsbad, USA). Singlet oxygen sensor green (SOSG) were purchased from Invitrogen (Carlsbad, USA).

Methyl thiazolyl tetrazolium, Hoechst 33342 (100×), and propidium iodide (PI) were obtained from Beyotime Biotechnology (Haimen, China).

Besides, deionized (DI) water with a resistivity of 18.1 MΩ cm was used in all relevant experiments.

4.2. Instruments

Ultraviolet-visible (UV-vis) absorption spectra were recorded using a UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan) in a standard quartz cuvette with 1 cm path length. Scanning electron microscopy (SEM) images were captured by an Apreo S LoVac SEM at 2 kV (FEI, Hillsboro, USA). Transmission electron microscope (TEM) images were recorded using a JEM2100 instrument (JEOL, Tokyo, Japan). High-resolution (HR) TEM images and elemental mapping were acquired via a Talos F200X G2 instrument (FEI, Hillsboro, USA). Zeta potential values were measured via a laser light scattering spectrometer (NanoBrook 173plus and ZetaPals/BI-200SM, New York, USA). Infrared spectra were performed on a Fourier transform infrared (FT-IR) spectrometer (Nicolet AVATAR-360, ThermoFisher, USA). The dark-field microscopic imaging experiments were carried out using a Nikon Eclipse Ni-U upright optical microscope (Nikon, Tokyo, Japan) with a laser beamsplitter (20 × 20 mm, Edmund Optics, Barrington, USA). The images were collected by a high-resolution color microscope camera (Digiretina 16, Xintu Optoelectronics Co., LTD, Fujian, China). The trajectories of nanomotors were captured by a sCMOS camera (Orcaflash 4.0, Hamamastu, Japan). Furthermore, the polarization-dependent scattering signals of single nanomaterial were recorded with a rotating optical axis of the polarizer. Confocal fluorescent images were obtained with a confocal laser scanning microscope (CLSM, A1R+, Nikon, Tokyo, Japan). Temperatures were determined using an infrared temperature sensor (XINTEST HT-20, Guangzhou, China). The optical density (OD) values of blue oxidized TMB (oxTMB) and MTT was measured on a microplate reader (Sunrise, Tecan, Austria).

4.3. Abbreviations

JNMs: Janus nanomotors; GNC: gold nanocup; PtNPs: platinum nanoparticles; GNC-Pt: a nanozyme-powered cup-shaped nanomotor constituted of a GNC with PtNPs at the bottom; PbS NPs: octahedral PbS nanoparticles; POD: peroxidase; Tf: human transferrin; TfR: transferrin receptor; ICG: indocyanine green; mPEG-SH: methoxy polyethylene glycol thiol; GNC-Pt-ICG/Tf: GNC-Pt with the functionalization of Tf, ICG, and mPEG-SH; GNC-ICG/Tf: GNC with the functionalization of Tf, ICG, and mPEG-SH; ROS: reactive oxygen species; \(^{1}\text{O}_2\): singlet oxygen; CTAB: hexadecyl trimethyl ammonium bromide; TMB: 3,5,3',5'-tetrathiol benzidine; oxTMB: oxidized TMB; SOSG: singlet oxygen sensor green; PBS: phosphate buffer saline; DMEM: Dulbecco’s Modified Eagle Medium; PS: penicillin/streptomycin; FBS: fetal bovine serum; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SPT: single-particle tracking; SPR: surface plasmon resonance; UV-vis: ultraviolet-visible; SEM: scanning electron microscopy; TEM: transmission electron microscopy; HAADF-STEM: high-angle annular dark-field scanning transmission electron microscopy; FT-IR: Fourier transform infrared; MSD: mean-squared displacement;
NIR: near-infrared; PTT: photothermal therapy; PDT: photodynamic therapy; CLSM: confocal laser scanning microscopy.

4.4. Preparation of Nanozyme-Powered GNCs-Pt Janus Nanomotors

Firstly, Au selectively grew at one vertex of each octahedral PbS nanoparticles (PbS NPs), which was controlled by electron transfer from PbS to Au during Au nucleation (PbS@GNCs). Briefly, aqueous solutions of CTAB (0.68 mL, 0.1 M), acetic acid (HAc, 1.37 mL, 1 M), lead acetate (Pb(Ac)₂, 0.68 mL, 0.5 M), thioacetamide (TAA, 0.68 mL, 0.5 M), and DI water (10.75 mL) were mixed together at 25 °C. Then, the mixture was heated to 80 °C for 8 h. After reaction, the obtained PbS NPs were centrifuged (5000 rpm × 10 min) to remove the excessive precursors and redispersed into DI water (15 mL) [38]. Then, H₂PtCl₆ (0.8 mL, 1.0 mM) was added into as-prepared PbS@GNCs solution (15 mL), and stirred at 25 °C for 10 min. Then, AA (0.8 mL, 1 mM) was dropped slowly, and the mixture was heated to 90 °C for 3 h. The PbS@GNCs-Pt were obtained by centrifugation (5000 rpm × 5 min) and redispersed in CTAB solution (15 mL, 0.1 M). Thirdly, GNCs-Pt were prepared by selectively dissolving PbS components of PbS@GNCs-Pt by HCl. Briefly, HCl (0.75 mL, 5 M) was added into the PbS@GNCs-Pt solution (15 mL). Subsequently, the obtained solution was stirred at 65 °C for 12 h. The GNCs-Pt were finally obtained by centrifugation (5000 rpm × 5 min) and redispersion in DI water (15 mL).

4.5. Peroxidase (POD)-like activity

The POD-like activity of GNCs-Pt was conducted at room temperature in a 96-well plate using 3,5,3',5'-tetramethylbenzidine (TMB, 5.0 μL, 42 mM) as substrate. A series of different catalyzers (GNCs-Pt or GNCs) and concentrations of H₂O₂ (0, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0%, v/v) were added into the disodium hydrogen phosphate-citric acid buffer (0.1 M, pH 3.0), and the total volume of reaction systems were set to 210 μL in each well. The absorption of the reaction systems was monitored at 652 nm at certain time using a microplate reader, which were further drawn into a curve to determine the POD-mimetic activity of samples. In addition, the pH stability of GNCs-Pt was also evaluated by the above method in solutions with pH values in the range from 1.0 to 11.0 for 30 min.

4.6. Self-propulsion diffusion behavior analysis

The single-particle measurements were performed on a Nikon Eclipse Ni-U upright optical microscope. Taking GNCs-Pt for example, the GNCs-Pt were firstly immobilized on the pretreated glass slide surface (22 × 22 mm²). Then, the scattered light from the individual GNCs-Pt was measured with an objective (40x, numerical aperture (NA) = 0.75) and captured by a sCMOS camera (Orcaflash 4.0, Hamamastu, Japan. Pixel size 6.5 × 6.5 μm²). To measure the polarization-dependent scattering signal from individual GNCs-Pt, a polarizer was put below the oil dark-field condenser. Through rotating the optical axis of the polarizer (from 0 ° to 360 °), the orientation-dependent scattering signals from single GNCs-Pt were recorded by the sCMOS camera. All images were processed with ImageJ.

4.7. Motion behaviors

Monitoring the diffusion trajectories of nanoparticles in water at the single-particle level is a great challenge because of their fast 3D Brownian motion. On this account, glycerol (50%, v/v) was added to increase the viscosity of the medium and slow down the movement of GNCs-Pt in all relevant non-cell-tracking experiments.
Firstly, the GNCs-Pt were mixed with a series of different concentrations of H₂O₂ (0, 1, 2, 3, 5, and 10%, v/v) and glycerol. Subsequently, the mixture was injected into the chamber. The self-propulsion diffusion of GNCs-Pt in different conditions was observed by an objective (40×, NA=0.75). And each sample was videoed simultaneously for 10 s by a sCMOS camera (Orcaflash 4.0, Hamamastu, Japan. Pixel size 6.5 × 6.5 μm²) with a frame rate of 49.99 fps.

4.8. The fabrication of GNCs-Pt-ICG/Tf

To fabricate an active transport platform based on GNCs-Pt for synergistic enhanced photo-dynamic/thermal therapy, we further decorate transferrin receptor (Tf), indocyanine green (ICG), mPEG-SH on GNCs-Pt. To decorate Tf on the surface of GNCs-Pt, Tf was functionalized with thiol group. NHS-PEG-SH (10 μL, 0.5 mg/mL) and Tf (63 μL, 5 mg/mL) was added into Tris HCl buffer (90 μL, pH 8.5, 10 mM). The Tf-PEG-SH solution (6.0 μM) was obtained after the mixture solution was shocked gently at 25 °C for 2 h.

Firstly, GNCs-Pt stock solution (1 mL) was centrifuged (5000 rpm × 5 min) to remove the extra CTAB in the solution and redispersed in DI water (50 μL). Subsequently, BSPP (50 μL, 1 mg/mL) was added and gently stirred at 25 °C for 3 h to substitute the CTAB on the surface of GNCs-Pt. The BSPP modified GNCs-Pt was obtained by centrifugation (5000 rpm × 5 min) and redispersion in DI water (100 μL) [42, 44]. Then, Tf-PEG-SH solution (3 μL) was gradually added and the mixture was gently stirred for additional 3 h. After that, PEI (1 μL, 0.5 mg/mL) was added to endow GNCs-Pt with positive charge for loading ICG. After the addition of free ICG (7 μL, 0.5 mg/mL) for 6 h, mPEG-SH (5 μL, 0.5 mg/mL) was added and shocked for another 6 h to increase the stability and biocompatibility of the nanomotors. Finally, GNCs-Pt-ICG/Tf were collected by centrifugation (5000 rpm × 5 min) and suspended in DI water (100 μL).

As controls, a series of nanomaterials were prepared with the same methods, such as GNCs-Pt loaded with ICG (GNCs-Pt-ICG), GNCs-Pt decorated with Tf (GNCs-Pt-Tf), GNCs loaded with Tf and ICG (GNCs-ICG/Tf), and GNCs modified with Tf (GNCs-Tf, without ICG).

4.9. \( ^{1} \text{O}_2 \) generation capability assessment

The \( ^{1} \text{O}_2 \) production ability of GNCs-Pt-ICG/Tf was investigated with singlet oxygen sensor green (SOSG) as indicator [53]. All relevant experiments were conducted in PBS (pH 7.4, 10 mM) solution following pretreatment with nitrogen to avoid the interference from dissolved O₂ as much as possible. A certain of GNCs-Pt-ICG/Tf (0.27 mg/mL, 9.05 μM ICG loaded) was added into the mixture solution of SOSG (3 μM) and H₂O₂ (1%, v/v). Then, the mixture was irradiated with an NIR laser (808 nm, 2 W/cm²). The changes in fluorescence intensity were detected at predetermined intervals with a fluorescence spectrophotometer (Ex/Em = 470/527 nm). As controls, the \( ^{1} \text{O}_2 \) production ability in free ICG (9.05 μM) and GNCs-ICG/Tf (0.27 mg/mL) with different concentration of H₂O₂ and laser conditions were also evaluated.

4.10. Photothermal performance

To examine the photothermal conversion efficiency of GNCs-Pt-ICG/Tf, GNCs-Pt-ICG/Tf solution (1 mL) was added in a quartz cuvette and exposed to a NIR laser at a power of 2.0 W/cm² for 15 min. Then, the solution was cooled down naturally for
another 15 min. The temperature changes were recorded by an infrared thermal imaging camera every 30 s. The photothermal conversion efficiency ($\eta$) can be calculated according to Equation (1)[50]:

$$\eta = \frac{hS(T_{max sample} - T_{max H_2O} - Q_{dis})}{h(1 - 10^{-A_{808}})}$$  \hspace{1cm} (Equation 1)

where $h$ is the heat transfer coefficient, $S$ is the irradiated area, $T_{max sample}$ and $T_{max H_2O}$ are the maximum equilibrium temperature of the sample and $H_2O$, respectively. $T_{surr}$ is the ambient temperature of the surroundings ($T_{surr} = 30 ^\circ C$). $Q_{dis}$ means heat dissipation from the system to the surroundings, and it is calculated to be approximately equal to 0 mW. $I$ represents laser power (2.0 W/cm$^2$). $A_{808}$ is the sample absorbance at 808 nm.

When the heat input is equal to the heat output, $hS$ is calculated with the following Equation (2):

$$hS = \sum m_i C_{p,i} \tau_s \approx m_{H_2O} C_{H_2O} \tau_s$$  \hspace{1cm} (Equation 2)

where $m_{H_2O}$ and $C_{H_2O}$ are the mass and thermal capacity of the water, respectively. $\tau_s$, the heat dissipation time constant, is calculated by plotting a linear data of cooling period with the negative natural logarithm using the following Equation (3):

$$t = -\tau_s ln \theta = \tau_s ln \frac{T - T_{surr}}{T_{max} - T_{surr}}$$  \hspace{1cm} (Equation 3)

where $t$ is the cooling time (s).

$T_{max,GNCs-Pt-ICG/Tf} = 74.4 \ ^\circ C$, $T_{max,GNCs-ICG/Tf} = 73.3 \ ^\circ C$.

$Abs_{808 nm,GNCs-Pt-ICG/Tf} = 0.420$, $Abs_{808 nm,GNCs-ICG/Tf} = 0.472$.

Thus, according to experiments, the photothermal conversion efficiency ($\eta$) of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf under 808 nm laser (2.0 W/cm$^2$) are 44.31% and 41.09%, respectively.

**Photothermal stability:** The photothermal stability of GNCs-Pt-ICG/Tf solution was measured by cycle irradiation. Briefly, the solution was irradiated with 808 nm laser at 2.0 W/cm$^2$ for 10 min. Then, the laser was turned off, and the solution was cooled down to ambient temperature for another 10 min. The above procedures were repeated for 5 times, and the temperature changes were recorded by an infrared thermal imaging camera. As controls, the photothermal conversion efficiency and photothermal stability GNCs-ICG/Tf were also measured through the same methods.

### 4.11 Biological stability

PBS (pH 7.4, 10 mM) and DMEM were used to mimic the human blood plasma environments. GNCs-Pt-ICG/Tf was first mixed with different media ($H_2O$, PBS, and DMEM) for 30 min. Then, the biological stability of GNCs-Pt-ICG/Tf was investigated by a Nikon Eclipse Ni-U upright optical microscope.

**MTT assay:** The standard MTT cell assay was used to investigate the cytotoxicity of GNCs-Pt-ICG/Tf. Briefly, the HepG2 cells were first seeded in 96-well plates at a density of $4 \times 10^4$ cells per well and grown in 5% CO$_2$ at 37 $^\circ C$ for 8 h. The culture medium is DMEM with FBS (10%, v/v) and PS (1%, v/v). Then, the HepG2 cells were
incubated with GNCs-Pt-ICG/Tf at different concentrations (0, 1.0, 5.0, 10.0, 25.0, and 50.0 μg/mL) for another 24 h in dark. Subsequently, MTT solution (20 μL, 5 mg/mL) was added into each well. After 4 h of incubation, the culture medium in each well was abandoned and DMSO (200 μL) was added to each well. The absorbance at 492 nm was measured using a microplate reader.

As controls, the biological stability and dark cytotoxicity of GNCs-ICG/Tf, as well as the dark cytotoxicity of GNCs-Pt and GNCs (without the functionalization of Tf, ICG, and mPEG-SH) were also investigated.

4.12. Cellular uptake

HepG2 cells were seeded on a pretreated coverslip (22 × 22 mm²) in culture dishes at density of 1 × 10⁵ and cultured overnight. After the culture medium was abandoned, GNCs-Pt-ICG/Tf (50 μg/mL) was dispersed in DMEM and incubated with cells for additional 2 h. Then, the uninternalized nanoparticles were washed away with PBS solution (1 mL × 3 times). The uptake of GNCs-Pt-ICG/Tf by HepG2 cells was observed by a Nikon Eclipse Ni-U upright optical microscope. As controls, cell uptake of GNCs-Pt-ICG (without Tf) and GNCs-ICG/Tf (without PtNPs) was also investigated. Furtherly, to assess the specific recognition capability of GNCs-Pt-ICG/Tf, the cell uptake of GNCs-Pt-ICG/Tf and GNCs-ICG by NCTC1469 cells (a mouse fibroblasts cell line) was investigated.

4.13. Enhanced dual-modal phototherapy effect

The synergistic enhanced photo-dynamic/thermal therapy effect of GNCs-Pt-ICG/Tf was examined by confocal laser scanning microscopy (CLSM). Firstly, HepG2 cells were seeded in culture dishes at density of 8 × 10⁴ and cultured overnight. Then the cells were treated with GNCs-Pt-ICG/Tf for 24 h. The working concentration of GNCs-Pt-ICG/Tf was 50 μg/mL, which has been proved to be safe for living cells without 808 nm laser irradiation. After washing the residual nanoparticles with PBS and addition of the fresh culture medium, the cells were irradiated by 808 nm laser at 2.0 W/cm² for 10 min and incubated for another 24 h. Subsequently, all cells were stained with Hoechst 33342 (300 μL, 100×) and PI (500 μL, 0.03 mM) to distinguish living and dead cells before CLSM imaging.

As controls, the cell viability was also determined by cultivating HepG2 cells with GNCs-Pt-ICG/Tf (50 μg/mL) in dark, GNCs-Pt-Tf (50 μg/mL) upon 808 nm laser irradiation (2.0 W/cm²), as well as free ICG (1.66 μmol/L) upon 808 nm laser irradiation (2.0 W/cm²), respectively. And the corresponding cell mortality of HepG2 cells was calculated by the signal intensity ratio between PI channel and DAPI channel.

Acknowledgments

Author Contributions: X.W. and L.X. conceived the idea, designed and lead the experiments. X.W. synthesized, modified and characterized the nanoparticles, also tracked, characterized and analyzed the motion behaviors of different nanomotors. W.L. carried out the transmission electron microscope (TEM) characterization of different nanomaterials. X.W., Z.Y., S.L, and L.X. contributed to the paper writing and revision. L.X. supervised the project. All authors agreed with the final version.
**Funding:** This work was supported by the National Natural Science Foundation of China (NSFC, Project No. 22174079, 21974073).

**Conflicts of Interest**
The authors declare that there is no conflict of interest regarding the publication of this article.

**Data Availability**
All data needed of this study are available in the article and its Supplementary Information files.

**Supplementary Materials**
Section S1. Experimental section.
Section S2. Details of analytical data.
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Fig. S6. The average diffusion areas of GNCs-Pt with different H$_2$O$_2$ concentrations.
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Fig. S17. Characterization of GNCs-Pt-ICG/Tf.
Fig. S18. The loading capacity of ICG.
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Fig. S20. The photothermal effect of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf.

Fig. S21. Stability of GNCs-Pt-ICG/Tf in different media.

Fig. S22. Stability of CTAB-stabilized GNCs-Pt in different media.

Fig. S23. Cell viability of HepG2 cells after incubating with different nanomaterials.

Fig. S24. Stability of GNCs-ICG/Tf in different media.

Fig. S25. Stability of CTAB-stabilized GNCs in different media.

Fig. S26. The motion behaviors of GNCs-Pt-ICG/Tf and GNCs-ICG/Tf on HepG2 cell membrane.

Fig. S27. Uptake efficiency of GNCs-Pt-ICG/Tf and GNCs-Pt-ICG for NCTC1469 cells.

Table S1. $K_m$ and $V_{max}$ of GNCs-Pt towards TMB and H$_2$O$_2$.

Table S2. Fitting the curves of TE-MSD versus the time interval ($\Delta$t) of GNCs-Pt with different H$_2$O$_2$ concentrations.

Table S3. FT-IR analysis of GNCs-Pt-ICG/Tf (mPEG-SH) and GNCs-ICG/Tf (mPEG-SH).

References


