Assembly-Enhanced Indocyanine Green Nanoparticles for Fluorescence Imaging-Guided Photothermal Therapy

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Supplementary Information

Materials and methods

Chemicals

Z-His-NH_2 (ZHN) and Z-His-$NHNH_2$ (ZHNN) were purchased from Bachem (Bubendorf, Switzerland). Indocyanine green (ICG) and propidium iodide (PI) dyes were purchased from Sigma Aldrich Chemical Company. Cell culture media (Dulbecco's Modified Eagle Medium, DMEM) and heat-inactivated fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS), trypsin-EDTA, and penicillin−streptomycin were purchased from BioLegend Co.. Human breast adenocarcinoma cancer cell line was provided by Chinese Academy of Medical Sciences. Other materials were purchased from Beijing Chemical Co. Ltd unless otherwise noted.

Preparation of ZHN/ICG NPs and ZHNN/ICG NPs

Typically, ZHN/ICG NPs were prepared in the following steps. 10 μL ZHN DMSO solution was added to 988 μL ICG aqueous solution, in which mixed with 2 μL 1 M HCl aqueous solution as to adjust the pH value to 6.5 approximately, where the final concentration of ZHN and ICG was 1 mg mL^{-1} and 0.2 mg mL^{-1}, respectively. The obtained colloid solution aged for 24 h, and then the above ZHN/ICG NPs were centrifuged in the instrument (9391 RCF, Eppendorf Centrigue 5424) at the speed of 10000 rpm for 20 min twice. Finally. the ZHN/ICG NPs were re-dispersed with ultrapure water.

The ZHNN/ICG NPs were prepared and treated with the same above method.

Characterization of NPs

The hydrodynamic diameter, ζ potential and distribution of ZHN/ICG NPs and ZHNN/ICG NPs were measured by a ZetaSizer Nano ZS (Malvern Instruments, ZEN 3600). S-4800 (Hitachi, Japan) with 10 kV accelerating voltage was used for scanning electron microscope (SEM) observation.

The entrapment of targeted components was determined by calibration curves with a range of known standard concentrations. The component of ZHN was analyzed by a high-pressure liquid chromatography (Thermo Scientific UltiMate 3000), and the component of ICG was analyzed by an absorption spectrophotometer (Shimadzu UV-2600). The entrapment efficiency value was calculated by the following formula (1):

$$\text{Entrapment of ef}\mathit{f}\text{iciency} = \frac{\text{weight of targeted components in the precipitate}}{\text{weight of } \mathit{targeted\ components} \text{ added}} \times 100\% \quad (1)$$

Transmission electron microscope (TEM) images were performed with a JEOL JEM-1011, operated at an acceleration voltage of 100 kV, for which samples were carefully placed onto the carbon coated copper grids. The spectrophotometer with a quartz cuvette of 1 mm path length was used for determination of the absorption data and a Hitachi F-4500 fluorescence spectrometer equipped with Xenon lamp as excitation source was used to record the fluorescence spectra.

Measurement of photothermal performance

Free ICG and ZHN/ICG NPs (1.0 mL) with different concentrations (0.025 mg·mL^{-1}, 0.05 mg·mL^{-1} and 0.20 mg·mL^{-1}) were put in a quartz cuvette and irradiated by the 808 nm laser at a power density of 2 W cm^{-2} for 10 min at room temperature (25 °C). Pure water was used as a control group. The temperature was recorded every 1 s with a digital thermometer. Cyclic photothermal conversion stability was test in a temperature increase- and decrease mode. Per-cycle of circulation time was 20 min. The total circulation times up to 3.

The photothermal conversion efficiency (η) of the free ICG and ZHN/ICG NPs was calculated following a standard formula (2):

$$\eta = \frac{hA\left(T_{max} - T_{sur}\right) - Q_{diss}}{I\left(1 - 10^{-A}\right)} = \frac{mc\left(T_{max} - T_{sur}\right) - Q_{diss}}{\tau_s I\left(1 - 10^{-A}\right)} \quad (2)$$

"T_{max}" and "T_{sur}" were initial and the highest temperature of free ICG and ZHN/ICG NPs. "Q_{diss}" was measured by a Spectra-Physics power meter, representing the heat dissipation. "I" denoted the power of laser. "A" was the absorbance at 808 nm. "m" denoted the quality of tested solution and "c" is the specific heat capacity of water. The value of "τ_s" was calculated by the following formula (3):

$$\tau_s = -t/\ln\theta \quad (3)$$

"θ" is the dimensionless driving force and "t" is the corresponding time.

In vitro evaluation of ZHN/ICG NPs

Human breast adenocarcinoma cancer cells (MCF-7 cells) were cultured in DMEM containing 10% FBS at 37 °C in humidified ambiance of 5% CO_2. For cellular uptake test, MCF-7 cells were placed in a 35 mm glass-bottom Petri dish (6×10^4 cells cm^{-2}) and allowed to culture for 24 h. ZHN/ICG NPs with different final concentration (0.006 mg mL^{-1} – 0.3 mg mL^{-1}) were added to 96-well plates. MCF-7 cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for further incubating 24 h. The cells were then washed with fresh culture medium and irradiated by the 808 nm laser (1.5 W cm^{-2}, 1 min). After irradiation, the cells were incubated in dark atmosphere for another 24 h before the viability test by the MTT method according to the manufacturer's protocol.

To test the in vitro photo-stability, the cells were cultured and placed with same above procedures. Then, ZHN/ICG NPs with the concentration of 0.025 mg mL^{-1} were added to 96-well plates for 24 h. For the first photo-irradiation treatment, the cells were washed with fresh culture medium and irradiated once by the 808 nm laser (1.5 W cm^{-2}, 1 min). For the second photo-irradiation treatment, the cells were washed and irradiated twice.

In vitro confocal laser scanning microscopy (CLSM) images were obtained as follows. MCF-7 cells were seeded at a density of 5×10^3 cells in Petri dishes and incubated for 24 h at 37℃. ZHN/ICG NPs with the concentration of 0.15 mg mL^{-1} were added to the dishes and for another 24 h. The cells were irradiated by the 808 nm laser (1.5 W cm^{-2}, 1 min) and then they were washed three times with PBS and then fixed with 4% paraformaldehyde. 10 μL PI dyes was added to the dishes as to indicate the apoptosis of cells. Imaging was monitored using CLSM (Olympus FV1000). The mean fluorescence intensity of PI was analyzed by the equipped software.

In vivo evaluation of ZHN/ICG NPs

Animal procedures were approved by the Ethics Committee of the Institute of process engineering, Chinese Academy of Sciences (permit number: IPEAECA2018061).

The mice tumor models were established as follows. Female BALB/c-nude mice (Beijing HFK Bioscience Co. Ltd.) were kept in an environmentally controlled animal facility with regular 12/12 cycle. The human xenograft model was prepared by inoculating 100 μL MCF-7 cell suspension at a concentration of 5.0×10^7 cells mL^{-1} to the sub-dermal dorsal area of mice. After that, the tumor volume was recorded and calculated by the following formula (4):

$$\text{Tumor volume} = \frac{length \times width \times width}{2} \times 100\% \quad (4)$$

Approximately 1 week after inoculation, the mice xenograft MCF-7 tumor models were well-established.

For *in vivo* fluorescence imaging, the mice bearing MCF-7 xenograft tumors were intratumorally injected with ZHN/ICG NPs (1.5mg mL^{-1}, 50 μL). At different time intervals (0 h- 48 h), the whole-body fluorescence imaging of mice was performed in the system (FX Pro, Kodak, Japan). Acquired images were analyzed by the equipped Living Imaging software.

For *in vivo* photothermal therapy, the mice bearing MCF-7 xenograft tumors were intratumorally injected with PBS (50 μL), free ICG (1.5 mg mL^{-1}, 50 μL) and ZHN/ICG NPs (1.5 mg mL^{-1}, 50 μL), respectively. After 4 h post-injection, the mice tumors were irradiated by the 808 nm laser (0.8 W cm^{-2}, 10 min). An IR thermal imaging camera (FLIR E60) was used to record the temperature of mice tumor. The mice tumor volumes were recorded during the whole observation period (21 days) and calculated by the formula (2), and the mice body weights were measured as well.

Statistical analysis

All experiments were repeated at least three times. Data are expressed as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance (ANOVA), with $P < 0.05$ considered to be statistically significant.

Figures and tables

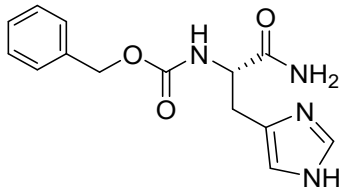

Fig. S1 Chemical structure of ZHN.

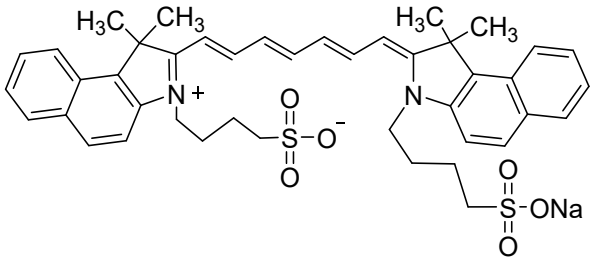

Fig. S2 Chemical structure of ICG.

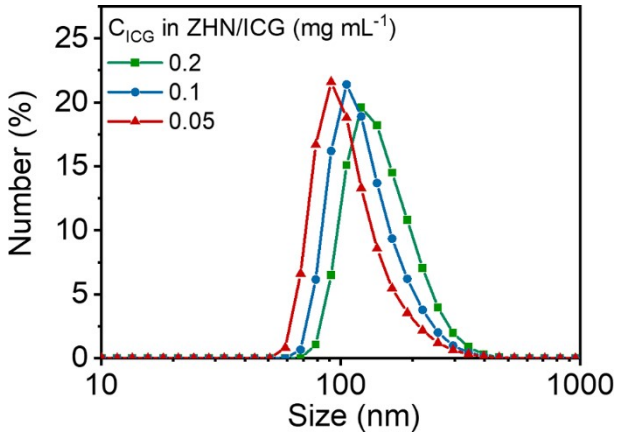

Fig. S3 DLS measurements of ZHN/ICG with different ICG concentrations.

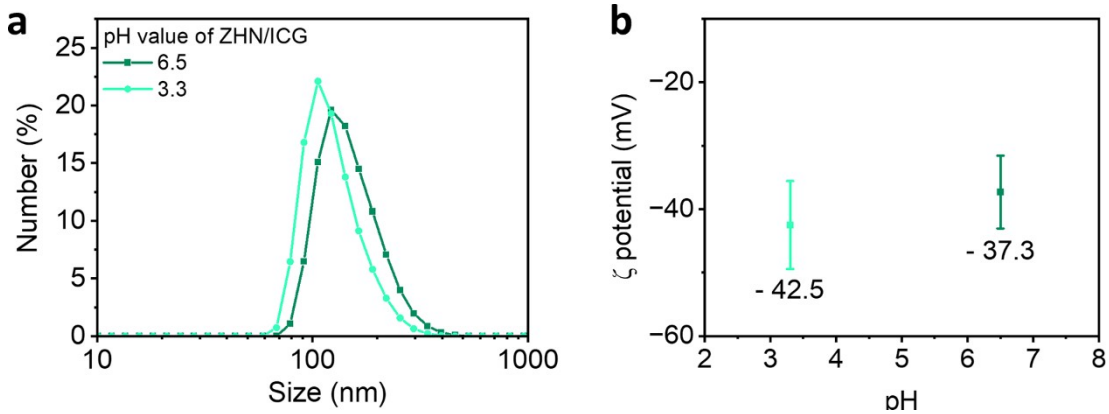

Fig. S4 DLS measurements of ZHN/ICG at different pH values: a) hydraulic diameter and b) ζ potential.

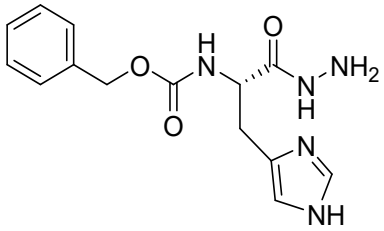

Fig. S5 Chemical structure of ZHNN.

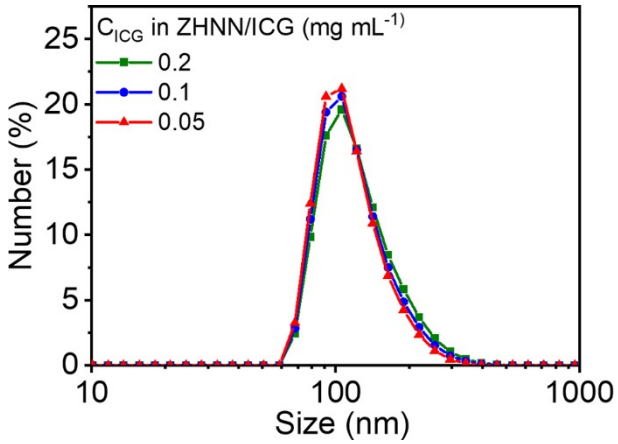

Fig. S6 DLS measurements of ZHNN/ICG with different ICG concentrations.

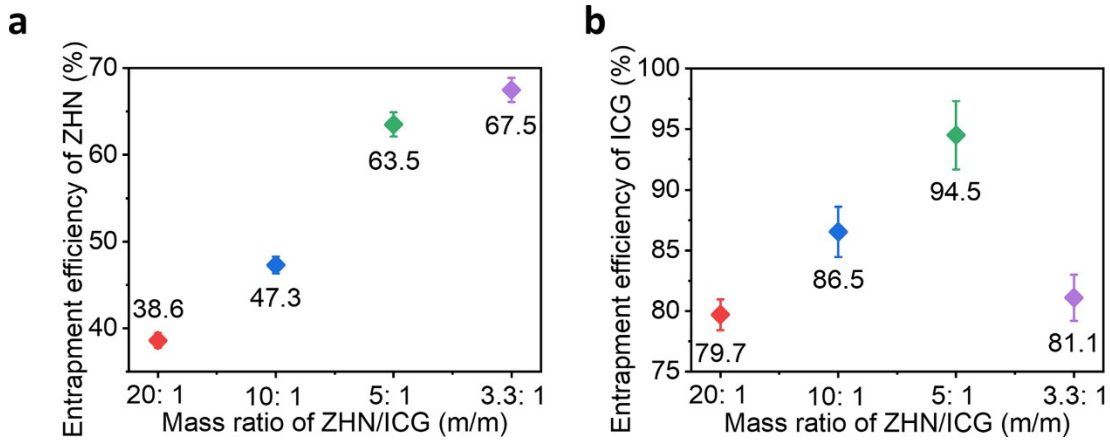

Fig. S7 Entrapment efficiency of a) ZHN and b) ICG in ZHN/ICG NPs at different mass ratios.

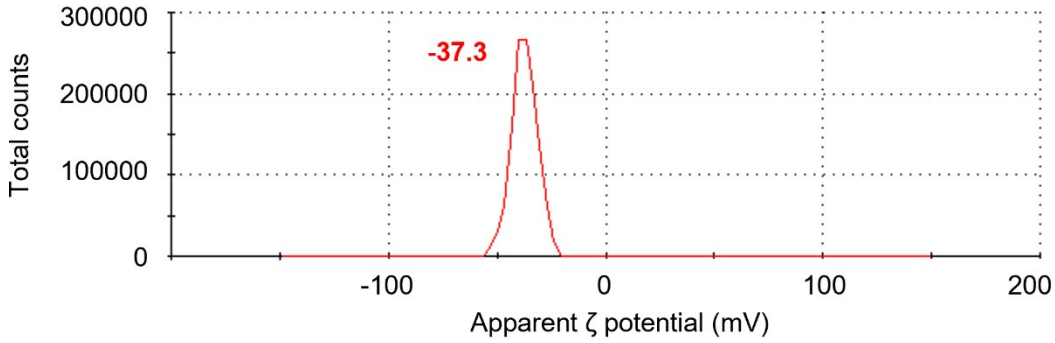

Fig. S8 ζ potential profile of ZHN/ICG NPs.

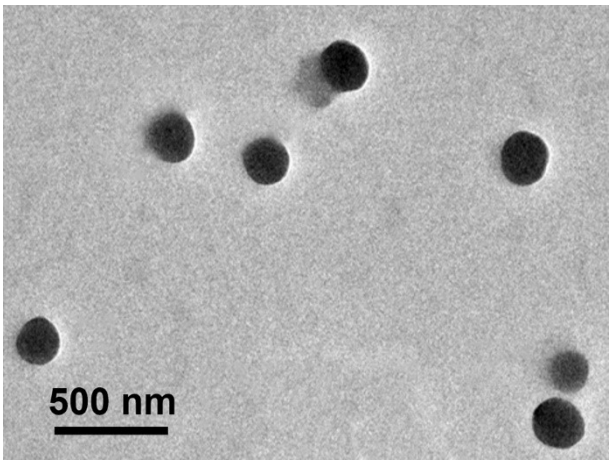

Fig. S9 TEM image of ZHN/ICG NPs.

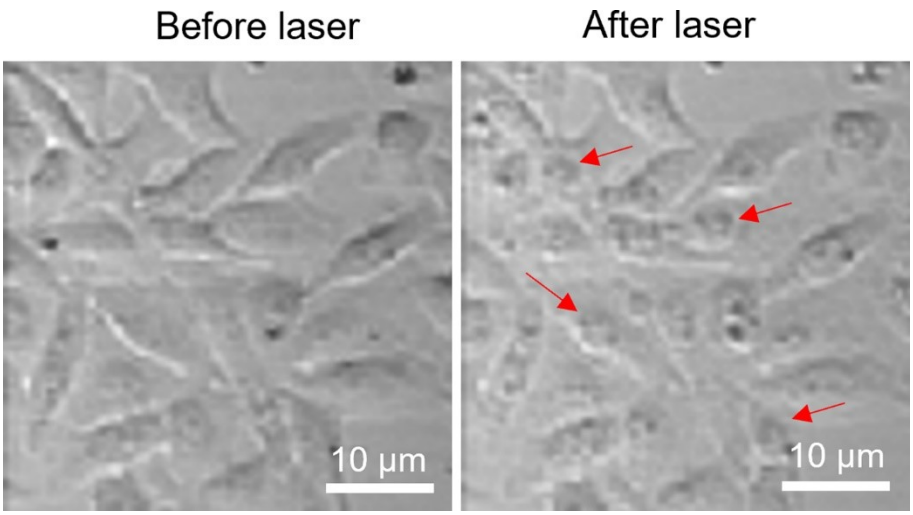

Fig. S10 Morphology change of MCF-7 cells before and after laser irradiation. Red arrows indicate the typical shrinking nucleus.

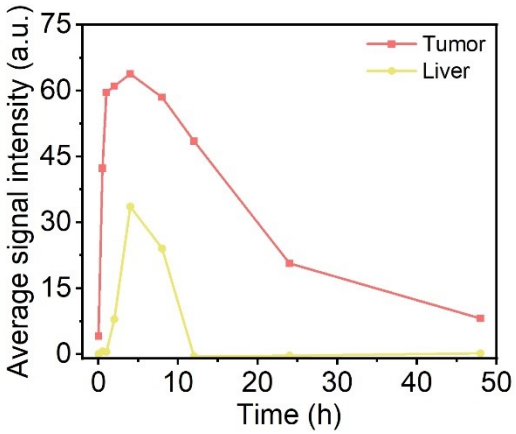

Fig. S11 The average fluorescence intensity in the tumor and liver as a function of time.

Tab. S1 DLS measurement data of ZHN/ICG with different ICG concentrations.

C_{ICG} in ZHN/ICG (mg mL^{-1})	Size (nm)
0.2	153.0±52.31
0.1	131.3±47.00
0.05	113.9±45.20

Tab. S2 DLS measurement data of ZHN/ICG at different pH values.

pH	Size (nm)	Z potential (mV)
6.5	153.0±52.31	-37.3
3.3	127.7±41.84	-42.5

Tab. S3 DLS measurement data of ZHNN/ICG with different ICG concentrations.

C_{ICG} in ZHNN/ICG (mg mL^{-1})	Size (nm)
0.2	127.7±48.80
0.1	122.5±44.81
0.05	118.2±40.08

Tab. S4 DLS measurement data of ZHN/ICG NPs at different conditions.

Treatment conditions	Size (nm)
Fresh ZHN/ICG NPs	153.0±52.31
Aged ZHN/ICG NPs	150.3±55.65
ZHN/ICG NPs + FBS + 37°C	169.6±59.55
ZHN/ICG NPs 5-fold dilution	149.7±61.61
ZHN/ICG NPs 10-fold dilution	152.6±50.93
ZHN/ICG NPs + 0.1M NaCl	263.4±109.1
ZHN/ICG NPs + 1M NaCl	1205±275.7