Supporting Information

Design of an Activatable NIR-II Nanoprobe for the In Vivo Elucidation of Alzheimer's Disease-Related Variations in Methylglyoxal Concentrations

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Materials and Methods

1. Materials

2-[2-[2-Chloro-3-[2-[1,3-dihydro-3,3-dimethyl-1-(4-sulfobutyl)-2H-indol-2-ylidene]ethylidene]-1-cyclohexen-1-yl]ethenyl]-3,3-dimethyl-1-(4-sulfobutyl)-3H-indolium inner salt sodium salt (IR783), poly(isobutylene-alt-maleic anhydride) (PIMA) and oleic acidwere purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Sodium oleate and bromotrimethylsilane (TMSBr) was purchased from Macklin Biochemical Co., Ltd (Shanghai, China). NH_2-PEG-OCH_3 and NH_2-PEG-NH_2 were obtained from biomatrik Inc. (Zhejiang, China). Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) provided $FeCl_3 \cdot 6H_2O$, ethylenediamine, methyl-β cyclodextrin, chlorpromazine and LY294002. Octadecene was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Diethyl 2-bromoethylphosphonate was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI), triethylamine (TEA), 1-hydroxybenzotriazole (HOBT), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were all purchased from J&K Scientific Ltd. Ltd. (Beijing, China). Methylglyoxal (MGO) was purchased from Alfa Aesar Chemical Co., Ltd. (China). Benzaldehyde (BA), formaldehyde (FA), glyoxal (GO), glyoxylic acid (GOA), o-phthalaldehyde (OPA), malondialdehyde (MDA) and ethyl pyruvate (EPRY) were purchased from Aladdin Industrial Corporation (Shanghai, China). Glyoxalase I (GLO1) inhibitor (ethyl (2S)-2-amino-5-[[(2R)-3-[(4-bromophenyl)-hydroxycarbamoyl]sulfanyl-1-[(2-ethoxy2-oxoethyl)amino]-1-

oxopropan-2-yl]amino]-5-oxopentanoate hydrochloride, BHGD) was purchased from MedChem Express LLC (Shanghai, China). GL biochem (Shanghai, China) supplied peptide Ac-EHAIYPRH-NH_2 (T7). DMEM medium, fetal bovine serum (FBS), penicillin-streptomycin solution and trypsin-EDTA solution were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). The ultrafiltration tube with a molecular weight cut-off of 30 KDa was purchased from Merck Millipore Co., Ltd. (Shanghai, China). The experiment was performed with water (18.2 MΩ cm) from a laboratory water purification system (Hitech Instruments Co., Ltd). The rest of the reagents without mentioned are of analytical grade.

2. Measurements and Characterization.

Cary 60 UV-Vis spectrophotometer and Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) were applied for the UV-Vis-NIR absorption and NIR-I fluorescence spectra measurement, respectively. The NIR-Ⅱ fluorescence spectra were measured by FS-5 fluorescence spectrometer in the 850-1500 nm region upon excition at 808 nm laser (Edinburgh Instruments, UK). The fluorescence lifetime of MAP was measured using a fluorescence lifetime spectrometer (QM40, Photon Technology International) with a 370 nm excitation source and the detector at 1050 nm. Malvern zetasizer Nano ZS at 25 °C (ZS90, Malvern, U.K.) was applied for the size distribution and zeta potential measurement. Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, USA) was used for fourier transform infrared (FTIR) spectra . The ^{1}H NMR spectra were measured through a Bruker Advanced Nuclear Magnetic Resonance Spectrometer (500 MHz). Transmission electron

microscope images (TEM) were measured by JEM-2100F transmission electron microscope (Electronics Co., Ltd., Japan). The flow cytometric analysis was obtained through Guava easyCyte flow cytometer (Merck Millipore, Massachusetts, USA) and BD FACSAria Ⅱ Flow cytometer (Becton,Dickinson and Company, USA). The MTT analysis was obtained through Cytation 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT, USA). The fluorescence images of the cells were adopted through Cytation 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT, USA) and a Leica TCS SP8 confocal laser scanning microscope (Leica, Germany) with a 63×oil objective lens and a 1.40 numerical aperture. The NIR-II imaging process was conducted through *in vivo* imaging system in the second nearinfrared window with an 808 nm diode laser, an 1000-nm long-pass filter with exposure time of 50 ms (PerkinElmer, USA). *In vivo* optical imaging system (IVIS, Caliper, USA) was applied for recording the NIR-I images. The concentration of Fe was determined by using inductively coupled plasma-optical emission spectrometer (ICP-OES, Agilent 720ES) and inductively coupled plasma-atomic emission spectrometer (ICP-AES, ThermoFisher ICAP7200HS). Mindray's BC-2800vet automatic hematology analyzer and Chemray240 automatic biochemical analyzer were used for recording whole blood hematology and serum biochemical analysis, respectively.

3. Preparation of Oleate-Fe_3O_4

Prepare a mixed solution of 80 mL ethanol, 60 mL H_2O and 140 mL hexane at first. $FeCl_3 \cdot 6H_2O$ (10.8 g, 40 mmol) and sodium oleate (36.5 g, 120 mmol) were dissolved

in the mixed solution, which was heated to 70 °C and stirred for 4 h. After extraction and washing with water, the upper organic layer was reserved. Then, the hexane solvent was removed by rotary evaporation to obtain a waxy solid form, termed iron-oleate complex. Freshly prepared iron-oleate complex (3.6 g) and oleic acid (3.39 g, 12 mmol) were dissolved in 25 mL of octadecene. Then the solution was heated to 310 ℃ under nitrogen protection for 30 min. The resulting solution was then cooled to room temperature, and finally, 500 mL of ethanol was added to the solution to precipitate nanoparticles.

4. Synthesis of NH_2-PAOEt

Diethyl 2-bromoethylphosphonate (1.8 mL, 10 mmol) was added dropwise to a 250 mL round bottom flask with 7.5 mL ethylenediamine (6.7 g, 100 mmol) at room temperature. Before adding 100 mL of ethyl acetate, the reaction mixture was further stirred for 2 h. The mixture was subjected to sonic degradation, allowed to stand for 30 min. After centrifugation, the upper ethyl acetate mixture was taken for vacuum rotary evaporation to remove ethyl acetate. The residue was purified by silica-gel column chromatography (chloroform/methanol = 10:1) to obtain a slightly yellow liquid with a yield of 89%.

5. Synthesis of PA-PIMA-PEG-NH_2

In a three-neck flask, PIMA (278.4 mg, 1.81 mmol) was dissolved in 1 mL of anhydrous dimethylformamide (DMF). The solution was stirred at 88 °C under nitrogen protection. Then NH_2-PAOEt (152.2 mg, 0.68 mmol) and NH_2-PEG-OCH_3 (350 mg, 0.68 mmol) dissolved in 0.5 mL of anhydrous DMF, were added dropwise to the above

solution. After stirring for 2 h, NH_2-PEG-NH_2 (82.1 mg, 0.15 mmol) dissolved in 0.5 mL of anhydrous DMF, was added dropwise to the above solution. The mixture were reacted under nitrogen protection for 72 h. The resulting solution was dialyzed against DMF and chloroform using 7000 Da dialysis bag. Finally, the solvent was removed to obtain a yellow gel-like solid, termed PAOEt-PIMA-PEG-NH_2.

TMSBr was added dropwise into 3 mL PAOEt-PIMA-PEG-NH_2 in dry dichloromethane in an ice bath. The reaction mixture was brought to room temperature and stirred overnight. After removing dichloromethane, 3 mL methanol was added to the residue. The mixture was stirred for another 8 h. Then methanol was removed to obtain a yellow solid, which was dialyzed against chloroform for 48 h. Finally, the solvent was removed to obtain a yellow gel-like solid, termed PA-PIMA-PEG-NH_2.

6. Ligand exchange

Typically, 2 mg of IONPs and 30 mg of PA-PIMA-PEG-NH_2 were dissolved in chloroform, mixed and stirred mechanically at 65 °C for 24 h. The mixture was then precipitated in hexane, centrifuged at 3900 rpm for 7 min. After filtered through a 0.45 μm syringe filter. Finally, the residue was centrifuged using an ultrafiltration tube with a molecular weight cut-off of 30 KDa (2500 rpm, 30 min) to obtain PA-IONPs.

7. Synthesis of Compound 2

Compound 1 was synthesized by previous literatures.[1, 2] Compound 1 (50 mg, 0.032 mmol) and iron powder (18 mg, 0.32 mmol) were dissolved with 2.5 mL acetic acid in a 25 mL Schlenk tube, followed by stirring at 100 °C for 2-3 h under argon protection. After extracting with ethyl acetate for three times and drying over with anhydrous

$MgSO_4$, the collected organic layer was concentrated in vacuo and purified by silica-gel column chromatography (petroleum ether/ethyl acetate = 1:2 v/v) to obtain compound 2 as brown solid with a yield of 79.1%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, J = 8.5 Hz, 3H), 7.36 (s, 3H), 7.13 (d, J = 8.3 Hz, 8H), 7.06 (d, J = 8.3 Hz, 14H), 4.21 (t, J = 7.5 Hz, 8H), 2.94 (t, J = 7.8 Hz, 8H), 2.63 (t, J = 7.8 Hz, 8H), 1.01 (t, J = 7.5 Hz, 8H), 0.07 (s, 36H). ^{13}C NMR (126 MHz, CDCl3) δ 174.57, 152.19, 149.04, 147.41, 147.04, 140.67, 136.95, 135.03, 131.16, 130.65, 129.11, 128.13, 126.15, 124.35, 123.83, 64.14, 54.89, 37.56, 31.83, 18.77, 1.46.

8. Synthesis of Compound 3 (MAM)

Compound 2 (50 mg, 0.033 mmol) were dissolved with 2.5 mL dichloromethane, followed by dropwise addition of trifluoroacetic acid (0.25 mL). The mixture was conducted at room temperature for 2 h, and then vacuum condensed to remove trifluoroacetic acid for the compound 3 (MAM) with a yield of 97%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.19 (s, 4H), 7.59 (d, J = 7.8 Hz, 4H), 7.49-7.42 (m, 4H), 7.31 (s, 4H), 7.19 (d, J = 7.5 Hz, 9H), 6.97 (d, J = 7.5 Hz, 9H), 2.79 (t, J = 7.0 Hz, 8H), 2.56-2.52 (m, 8H). ESI-QTOF MS: calcd for $C_{62}H_{53}N_6O_8S_3$ $[M+H]^+$ m/z 1105.3082; found 1105.3064.

9. Synthesis of Compound 4 (MAP)

Compound 3 (50 mg, 0.045 mmol) was dissolved in 2 mL DMF, followed by dropwise addition of 100 μL MGO solution (35–45% w/w a.q.). The mixture was conducted at room temperature for 2 h, and then vacuum condensed, purified by silica-gel column chromatography to get compound 4 (MAP) as green solid with a yield of 89.7%. ^{1}H

NMR (500 MHz, DMSO-d_6) δ 7.57 (s, 3H), 7.42 (q, J = 10.4 Hz, 3H), 7.34-7.26 (m, 2H), 7.18 (s, 10H), 6.97 (s, 10H), 2.82-2.77 (m, 8H), 2.54 (d, J = 9.2 Hz, 8H). ESI-QTOF MS: calcd for $C_{65}H_{53}N_6O_8S_3$ $[M+H]^+$ m/z 1141.3082; found 1141.306.

10. Synthesis of carboxylated IR783 dye

In a 50 mL round-bottom flask, 3-mercaptopropionic acid (39.2 μL, 0.45 mM) and triethyl amine (TEA, 62.3 μL, 0.45 mM) were add dropwise into IR783 (224.81 mg, 0.3 mM, 749.35 g/mol) anhydrous DMF solution. The mixture was stirred at room temperature for 24 h under nitrogen protection. The resulting solution was then washed with methyl tert-butyl ether and purified by a reversed-phase C-18 column to obtain a dark green product with a yeild of 78.5%.

11. Preparation of M-IONPs, TM-IONPs, I-IONPs and TI-IONPs

First, MAM (1150.3 g/mol, 10 mg, 9.047 μmol, 1.2 eqv), EDCI (191.7 g/mol, 8.8 mg, 45.235 μmol), HOBT (135 g/mol, 6.1 mg, 45.235 μmol) were dissolved in 500 μL dry DMF to activate carboxyl groups for 2 h. Then, TEA (5 μL, 45.235 μmol) and PA-IONPs (1.0 eqv) was added at 0 °C. After 24 h reaction at room temperature, the resulting solution was dialyzed against DMF and water using a dialysis bag with molecular weight cut-off 7000 Da. Finally, the residue was concentrated to a constant volume through an ultrafiltration tube (2500 rpm, 30 min) with a molecular weight cut-off of 30 KDa. The synthesis of TM-IONPs was followed as similar procedure.

To prepare I-IONPs, carboxylated IR783 dye (841 g/mol, 7.5 mg), EDCI (191.7 g/mol, 8.8 mg), HOBT (135 g/mol, 6.1 mg) were dissolved and stirred for 2 h. Then, TEA (5 μL) and PA-IONPs was added at 0 °C and stirred at room temperature for 24

h. Finally, I-IONPs was obtained through dialysis and concentration. The synthesis of TI-IONPs was followed as similar procedure.

12. Determination of payloads

The grafting ratio of MAM and carboxylated IR783 were determined using UV spectrophotometer and calculated throughout the corresponding calibration curves. The grafting ratio of T7 was calculated by improved Pauly chromogenic method for histidine through the corresponding calibration curves.

13. Cellular uptake

The bEnd.3 cells were seeded in 25 mm glass bottom dishes with a density of 5000 cells/well for 24 h and then incubated with methyl-β-cyclodextrin (MβCD, 1.9 mM), chlorpromazine (Ch, 2.9 μM) or LY294002 (LY, 2.9 μM) inhibitors for 30 min. After washing with PBS, the cells were treated with the same concentration of TI-IONPs (10 μM IR783) at 37 °C for 6 h. The group without inhibitor and the group only treated with I-IONPs (10 μM IR783) were used as controls. Then, the cells were washed with PBS and fixed with 4% paraformaldehyde for 15 minutes and imaged with a Cytation 3 cell imaging multimode reader (Cy 7 channel: λ_{ex} = 714 ± 40 nm, λ_{em} = 809 ± 81 nm). Additionally, the cells with different treatments were trypsinized, resuspended in PBS, and quantitatively analyzed by flow cytometric measurement.

14. NIR-II fluorescence imaging of endogeous MGO in SH-SY5Y cells

SH-SY5Y cells were seeded into a 24-well plate at a density of 1×10^4 cells/well for 24 hs. The cells were pretreated with glyoxalase I (GLO1) inhibitor (BHGD) for 6 h. Subsequently, the cells were incubated with TM-IONPs (30 μM MAM) for 2 h. Then,

the cells were washed, trypsinized and resuspended in PBS. The NIR-II fluorescence imaging was collected from 1000-1700 nm through NIR-II imaging system using a laser excitation wavelength at 808 nm with exposure time of 50 ms.

15. In vitro BBB transcytosis

Typically, bEnd.3 cells or hcMEC/D3 cells were incubated in the upper chamber of the transwell (No. 3401 Costar; Corning, 0.4 μm pore size and 1.13 cm^2 growth area) for 1 week at a density of 5×10^5 cells per chamber. Then 1×10^4 SH-SY5Y cells were seeded in the lower chamber and cultured for 24 h. Then TI-IONPs of 10 μM IR783 were added to the upper chamber and incubated for 12 h. Then the SH-SY5Y cells in the lower chamber were trypsinized, and collected. The group without bEnd.3 or hcMEC/D3 monolayer, the group incubated with IR783 or I-IONPs at the same concentration were used as the control groups. Finally, the SH-SY5Y cells in the lower chambers were collected for flow cytometry analysis and fluorescence imaging.

The transport percentage across the hcMEC/D3 monolayer was quantified by considering the fluorescence intensities of upper chamber at 0 h and at 12 h of the APC-Cy7 (IR783) flourescence channels, using a quation as follows:

The transport percentage (%)= $(F_0\text{-}F)/F_0 \times 100\%$, where F is the mean intensity of the signal within the ROI at 12 h, and F_0 is the initial mean fluorescence intensity at 0 h.

16. Animal model

Animal experiments were carried out in strict accordance with the guidelines of the Animal Experimental Ethics Committee of East China Normal University and approved by the committee. C57B/L6 mice (male; 25-30 g body weight; age, 7 months old) and

BALB/c nude mice (female; 13-15 g body weight; age, 5-weeks old) were obtained from Laboratory Animal Center of East China of Normal University (Shanghai, China). SD rats (female, 180 ± 10 g body weight; age, 4–5 weeks old) were purchased form Shanghai Experimental Animal Center (Shanghai, China). APPswe/PS1dE9 transgenic mice (male; 28-32 g body weight; age, 7 months old) were purchased from Jiangsu ALF Biotechnology Co., LTD (Jiangsu, China).

17. Biodistribution and pharmacokinetics profile *in vivo*

BALB/c nude mice were randomly divided into two groups as TI-IONPs and I-IONPs. Then the mice were followed by i.v. administration of TI-IONPs and I-IONPs (140 μmol Fe kg^{-1} body weight), respectively. The fluorescent images at different time (λ_{Ex} = 745 nm and λ_{Em} = 820 nm) were obtained using a Caliper IVIS Lumina II imaging system (PerkinElmer, USA). After that, the mice were sacrificed and the brains as well as major organs (i.e., heart, liver, spleen, lung, and kidney) were collected and examined for fluorescence imaging *ex vivo*. Moreover, the brains were frozen sectioned immediately and stained with DAPI after IVIS image, and then examined by using Cytation 3 cell imaging multimode reader. In addition, Prussian blue staining was applied to indicate the presence of Fe. Nissl staining sections were used for quantitative analysis of neuronal injury in the cortex of mice.

To investigate the pharmacokinetics of the Fe_3O_4 nanoparticles, SD rats were intravenously injected with TI-IONPs or I-IONPs solution at an identical dose of 140 μmol Fe kg^{-1}. Blood samples were collected at 15, 30 min, 1, 3, 6, 9 and 12 h

postinjection. The blood concentration of Fe was quantitatively examined using ICP-OES.

18. *In vivo* NIR-II fluorescence imaging of brain

First, the APP/PS1 mice were administrated by intravenous injection of 100 μL TM-IONPs or I-IONPs (140 μmol Fe kg^{-1} body weight). The group of wide-type mice injected with TM-IONPs weas used as control. The NIR-II images of mice anesthetized with isoflurane were recorded at various time points post-injection through NIR-II *in vivo* imaging system (MARS). After 120 min, the mice were euthanized, and brains were harvested for NIR-II images. The excitation wavelength was 808 nm, and the NIR-II fluorescence signal wass collected between 1000-1700 nm with exposure time of 50 ms.

19. Biosafety assay

PBS, TM-IONPs and M-IONPs solutions (140 μmol Fe kg^{-1} body weight) were intravenously injected into healthy BALB/c mice. The treatment was repeated triplicate at a time interval of 2 days. One week after injection, the mice were sacrificed and their blood was collected. An automatic hematology analyzer and an automatic biochemical analyzer were used to obtain whole blood hematology and serum biochemical analysis data.

For histopathological studies, at the end of treatment, major organs including heart, liver, spleen, lung, kidney and brain of each group mice were fixed in 4% paraformaldehyde immediately following sacrifice and stained with hematoxylin and eosin (H&E).

20. Statistical Analysis

Data were presented as the means ± standard deviations (SD) of at least three parallel samples. The significance of the difference was determined using one-way analysis of variance (ANOVA) with Tukey's post hoc test and log-rank (Mantel–Cox) test (*P < 0.05, **P < 0.01, ***P < 0.001).

Reference

[1] Y. P. Fang, Y. B. Huang, P. C. Wu, and Y. H. Tsai, *Eur. J Pharm. Biopharm.,* 2009, **73**, 391.

[2] D. K. Roper, W. Ahn and M. Hoepfner, *J. Phys. Chem. C.,* 2007, **111**, 3636-3641.

Tables and Figures

Table S1. Mole ratio of MEMCA and PA-IONPs, grafting ratio of MEMCA, size, PdI and zeta potential of M-IONPs.

Samples	**Mole ratio**		**Grafting ratio (%)**	**Size (nm)**	**PdI**	**Zeta potential (mV)**
	MAM	PA-IONPs	MAM	M-IONPs		
1	0.6	1	14.1%	24.40 ± 0.75	0.19 ± 0.00	-20.0 ± 1.7 mV
2	1.2	1	68.5%	24.40 ± 0.51	0.21 ± 0.01	-22.3 ± 0.8 mV
3	2.4	1	72.4%	28.20 ± 1.60	0.24 ± 0.05	-24.0 ± 1.4 mV

Table S2. Mole ratio of T7 and PA-IONPs, grafting ratio of T7, size, PdI and zeta potential of different samples.

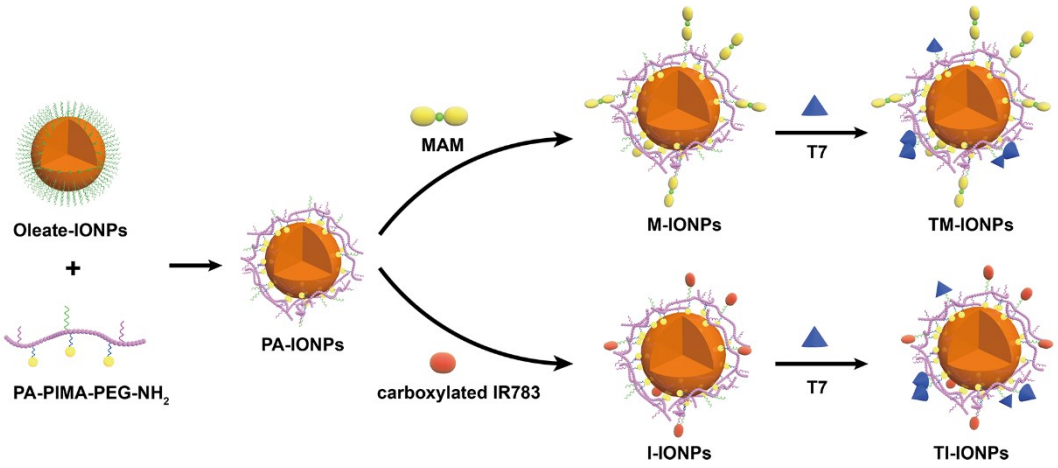
Samples	**Mole ratio**		**Grafating ratio (%)**	**Size (nm)**	**PdI**	**Zeta potential (mV)**
	T7	IONPs	T7	samples		
T7-IONPs	2.4	1	26.81%	24.40 ± 0.51	0.17 ± 0.02	-19.70 ± 0.2
TM-IONPs	2.4	1	18.10%	28.20 ± 0.62	0.22 ± 0.01	-22.10 ± 0.9

Table S3. Whole blood hematology assay of the BALB/c nude mice treated with PBS, TM-IONPs, and M-IONPs, respectively.

	WBC (10^9/L)	**RBC** (10^{12}/L)	**HGB** (g/L)	**HCT** (%)	**MCV** (FL)	**MCH** (pg)	**MCHC** (G/L)	**PLT** (10^9/L)
Reference	0.80~6.80	6.36~9.42	110~143	34.6~44.6	48.2~58.3	15.8~19.0	302~353	450~1590
PBS	1.60±0.26	7.89±0.65	127±6	38.7±3.6	48.9±0.7	16.1±0.6	329±16	632±26
TM-IONPs	1.50±0.10	8.05±0.54	128±8	40.5±2.5	50.3±0.2	15.9±0.2	317±3	769±31
M-IONPs	1.33±0.37	7.25±0.58	115±8	35.6±3.5	49.2±2.2	15.9±0.5	324±8	699±20

Table S4. Serum biochemistry assay of BALB/c nude mice treated with PBS, TM-IONPs, and M-IONPs, respectively.

	ALT (U/L)	AST (U/L)	BUN (mg/dL)
Reference	10.06-96.47	36.31-235.48	10.81-34.74
PBS	53.01±2.22	116.96±24.25	21.84±2.99
TM-IONPs	54.52±6.16	143.43±26.75	21.51±1.85
M-IONPs	46.95±1.42	128.98±20.44	20.65±4.65



Scheme S1. Schematic illustration of the preparation of different formulations.

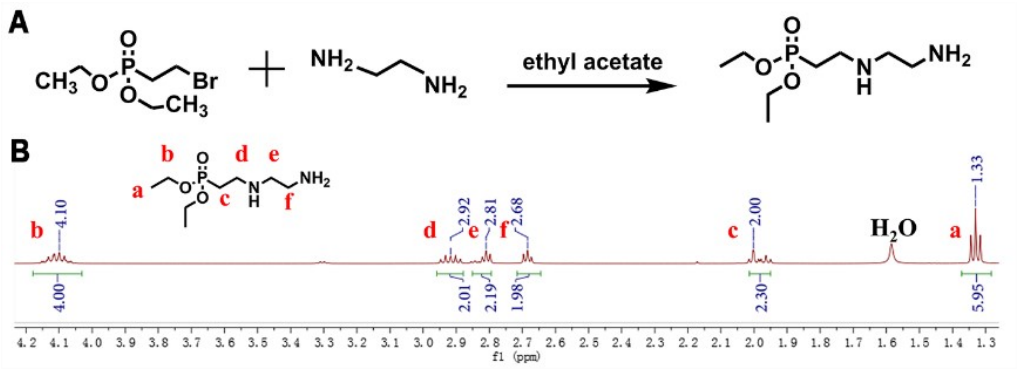


Fig. S1 (A) Schematic representation of the reaction. (B) ^{1}H NMR spectra of NH_2-PAOEt in $CDCl_3$.

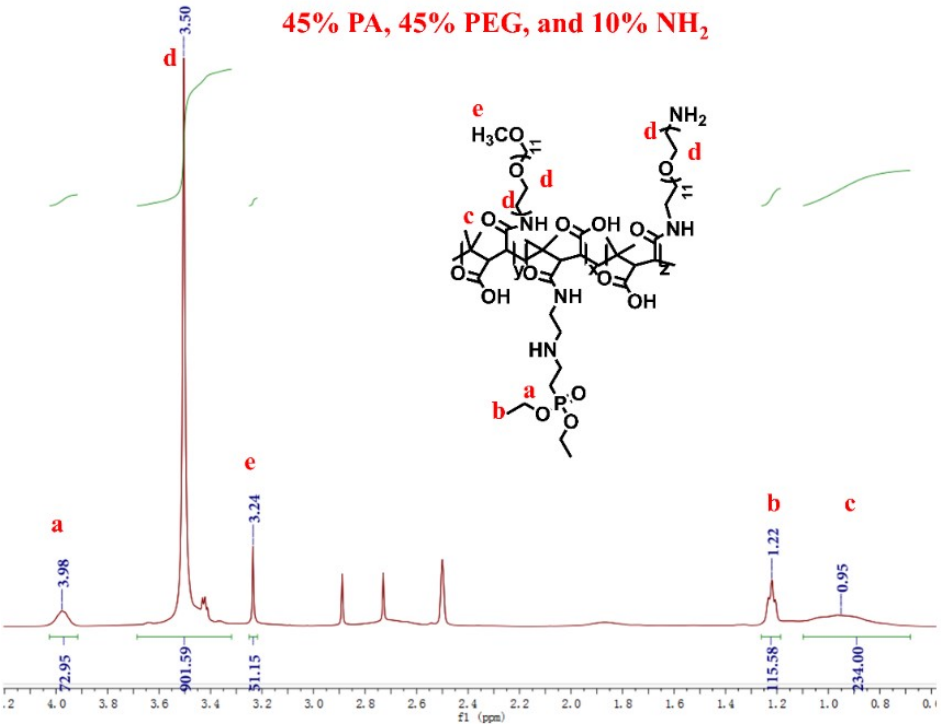


Fig. S2 ^{1}H NMR spectra of PAOEt-PIMA-PEG-NH_2 in DMSO-d_6 with the data of peak area integral.

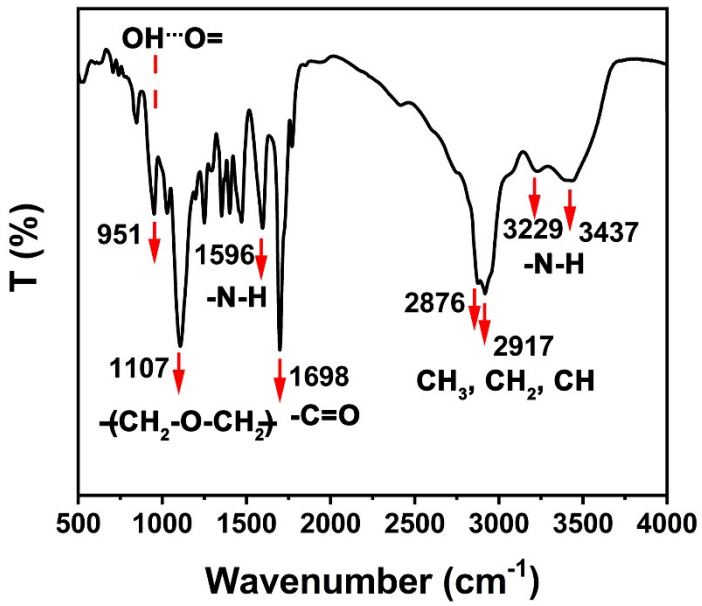


Fig. S3 FT-IR spectra of PA-PIMA-PEG-NH_2.

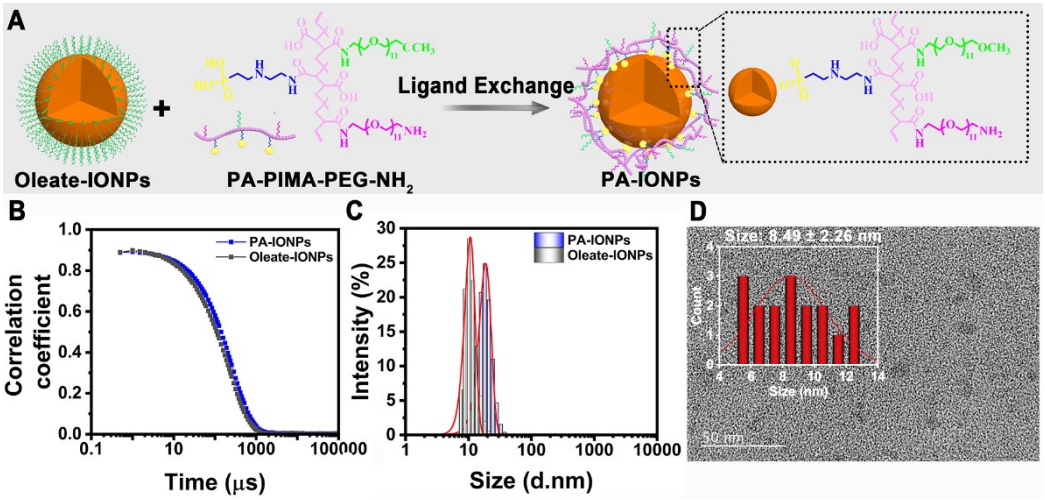


Fig. S4 (A) Schematic representation of the reaction. (B&C) Intensity correlation functions and size ditruibution collected from dispersions of PA-IONPs before and after phase transfer. (D) TEM image and the averaged size distribution of PA-IONPs.

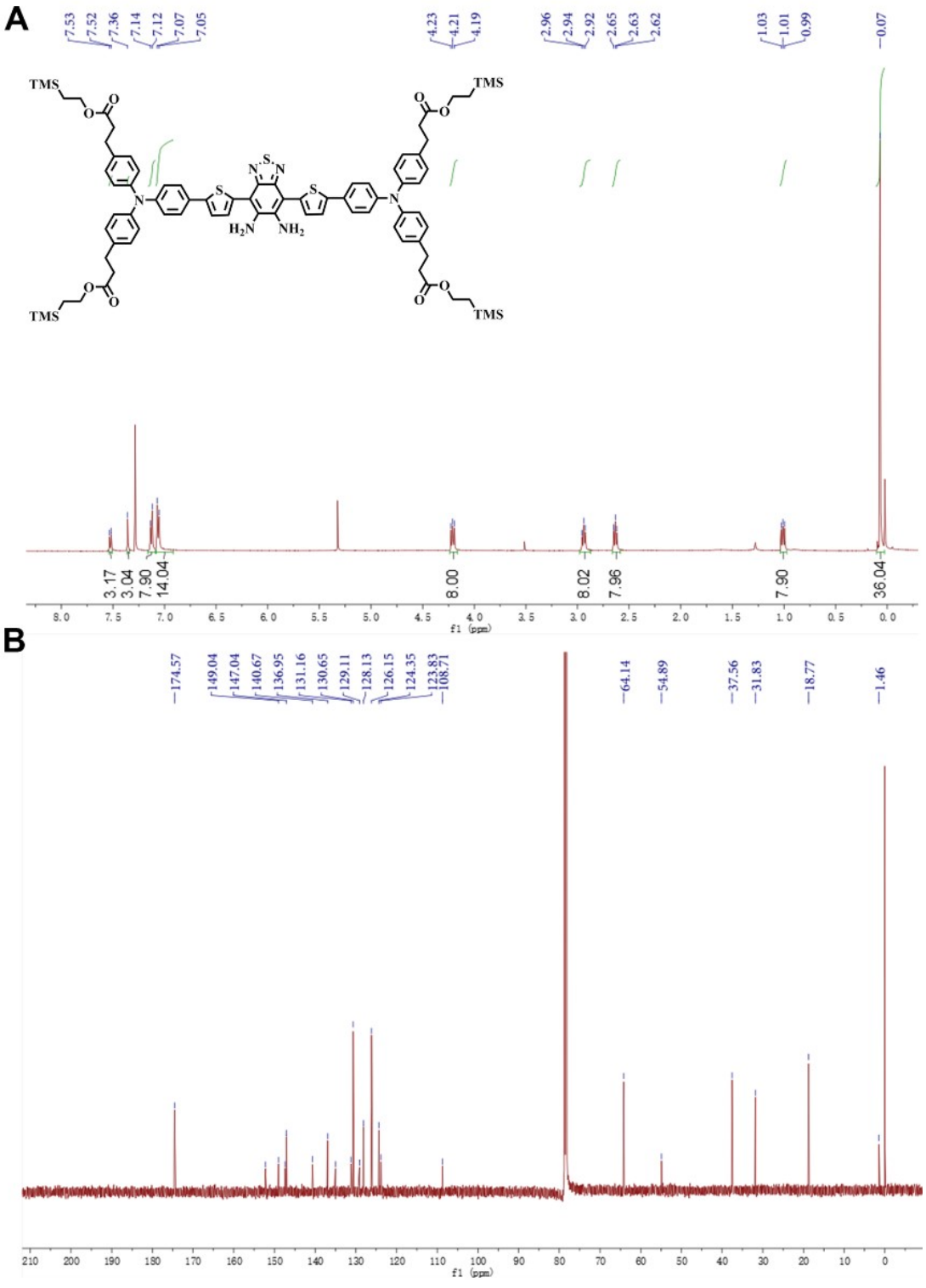
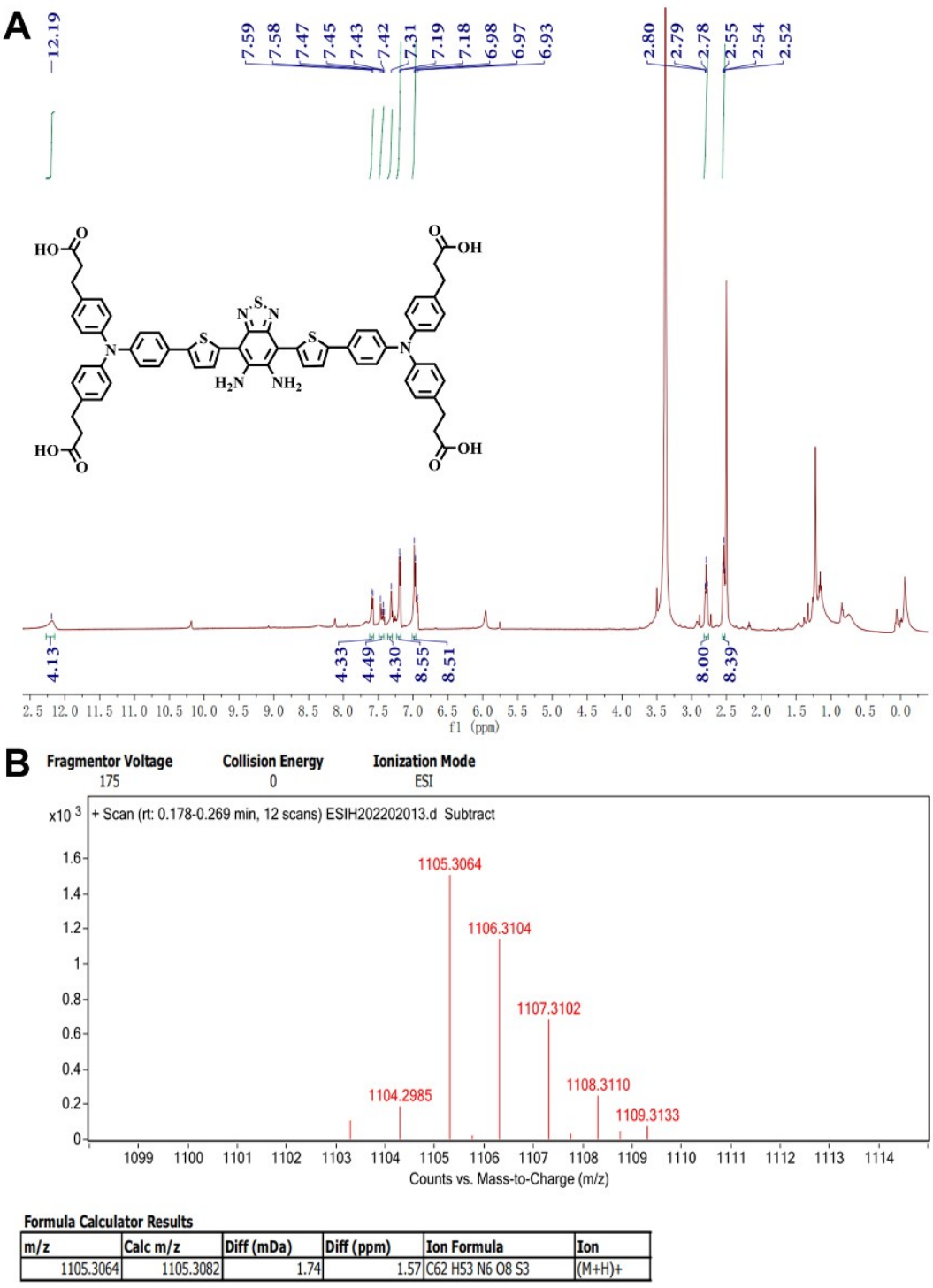


Fig. S5 (A) ^{1}H NMR and (B) ^{13}C NMR spetra of compound 2.



Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
1105.3064	1105.3082	1.74	1.57	C62 H53 N6 O8 S3	(M+H)+

Fig. S6 (A) ^{1}H NMR and (B) ESI-QTOF MS spectra of compound 3 (MAM).

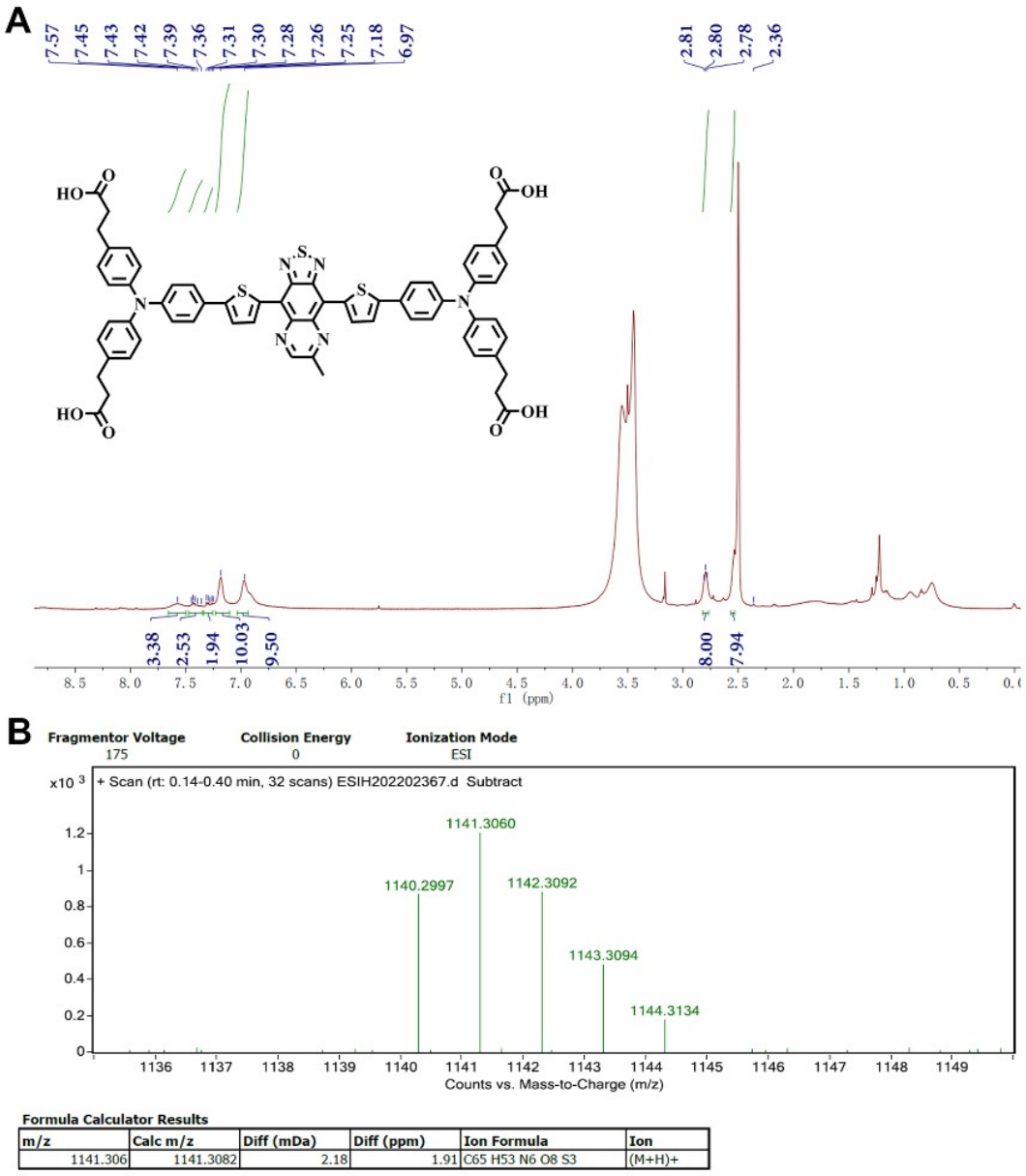


Fig. S7 (A) ^{1}H NMR and (B) ESI-QTOF MS spectra of compound 4 (MAP).

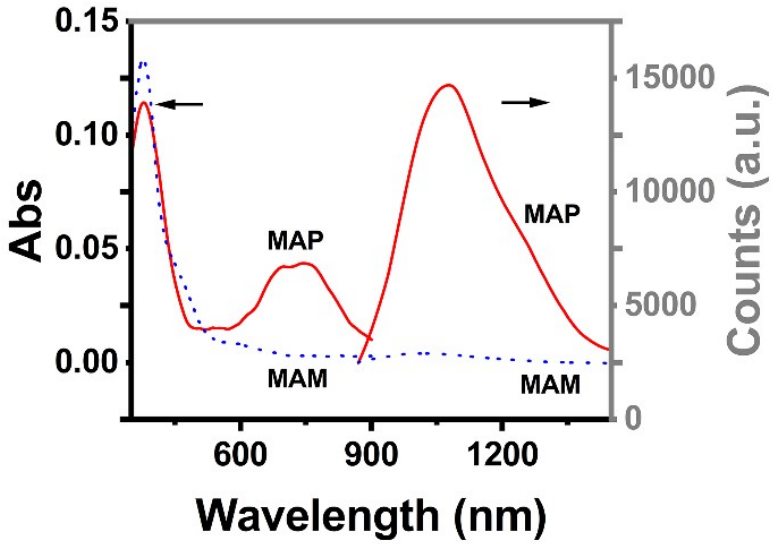


Fig. S8 The absorption spectra and NIR-Ⅱ fluorescence spectra of MAM and MAP.

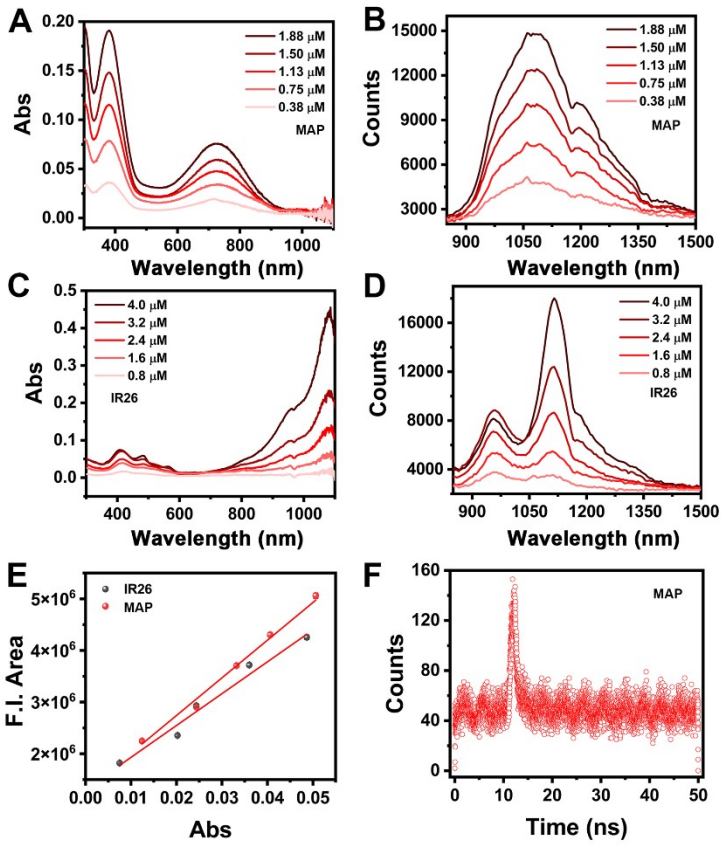


Fig. S9 (A-B) The absorbance and fluorescence spectra of different concentration of MAP in DMSO. (C-D) The absorbance and fluorescence spectra of different concentration of reference molecule IR26 in dichloroethane. All samples were tested upon the same excitation wavelength, slit width and voltage. (E) The relationship of integrated fluorescence area with absorbance. (F) Time-resolved transient spectra of MAP at the excitation wavelength of 370 nm.

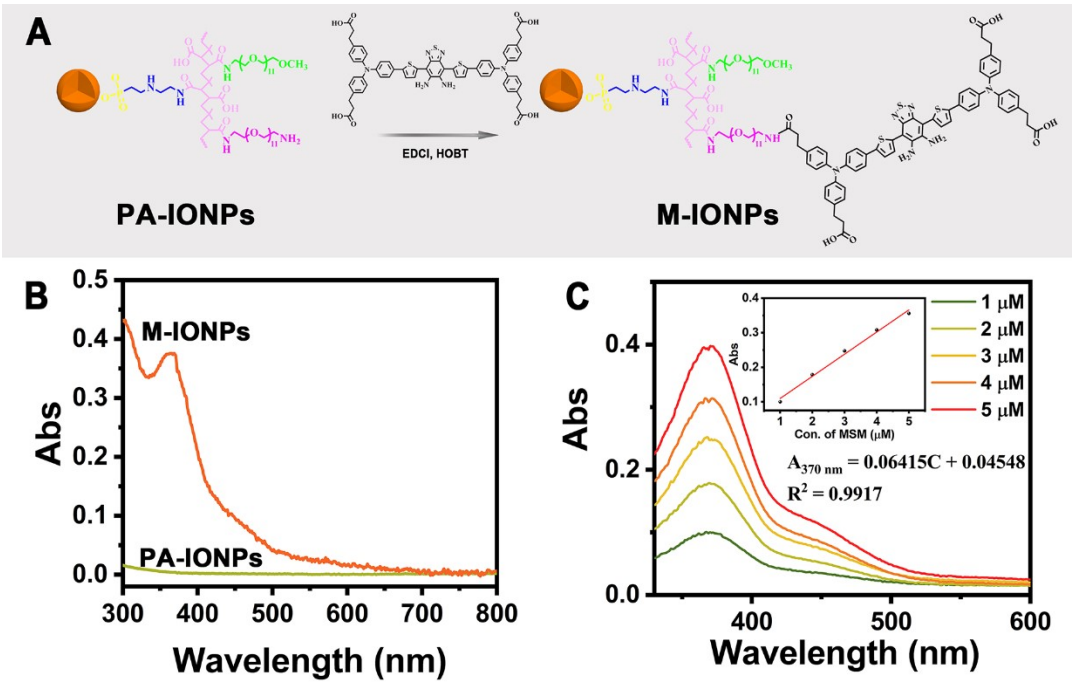


Fig. S10 (A) Schematic representation of the synthesis of M-IONPs. (B) Absorbance spectra of PA-IONPs and M-IONPs. (C) Absorbance spectra of MAM and corresponding calibration curve.

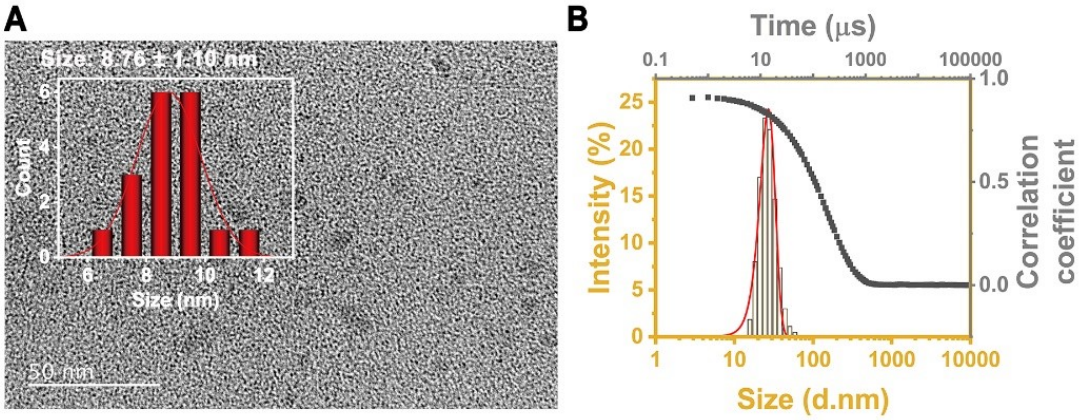


Fig. S11 (A) TEM image and the averaged size distribution of M-IONPs. (B) Intensity correlation functions and size distribution of M-IONPs.

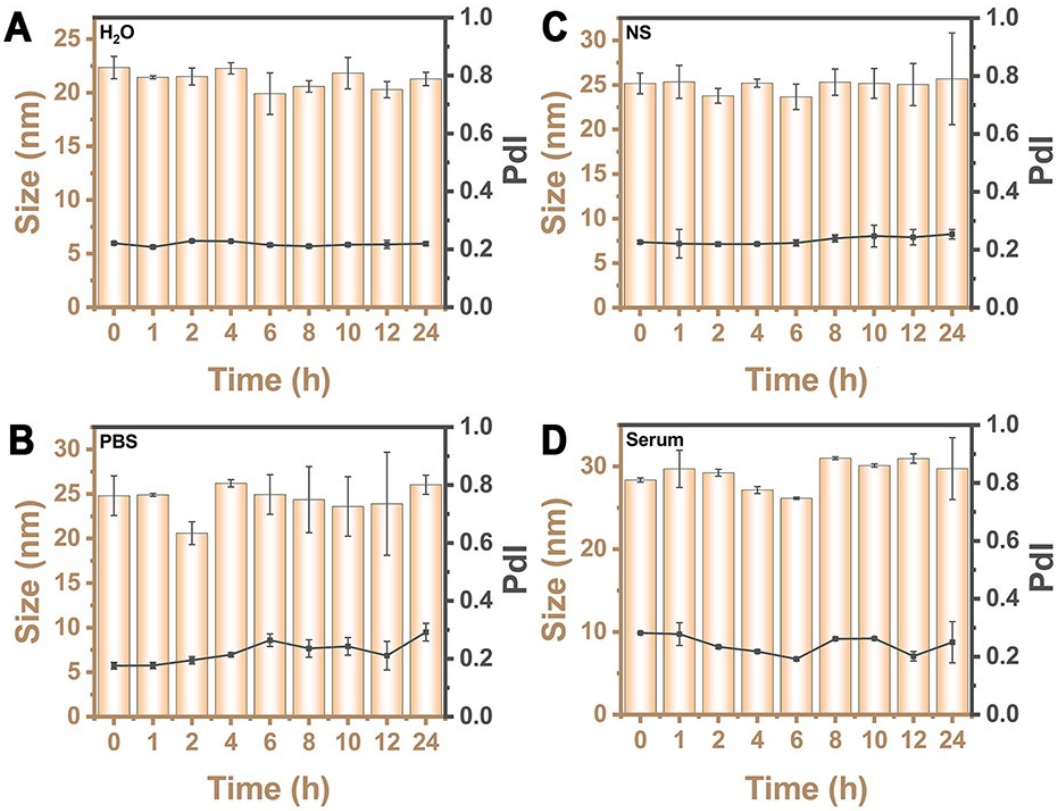


Fig. S12 Changes in size and PdI of M-IONPs in different media.

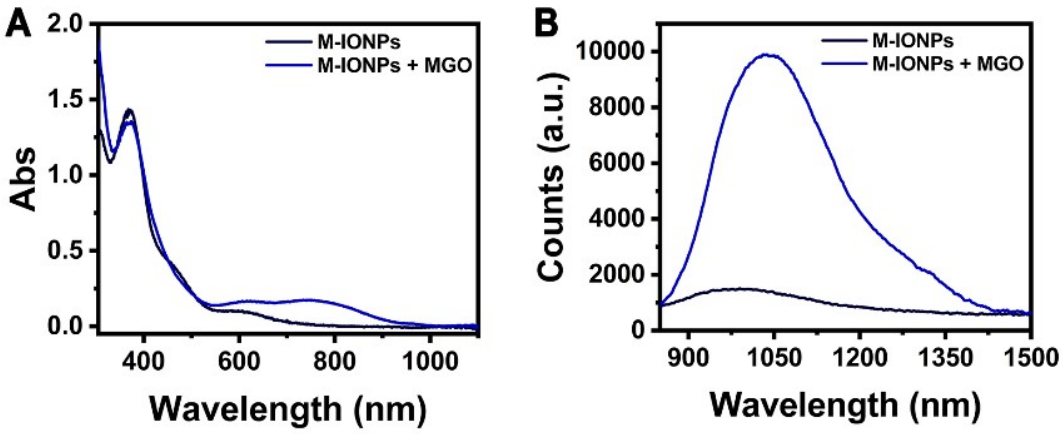


Fig. S13 Absorption and fluorescence spectra of M-IONPs with the addition of MGO.

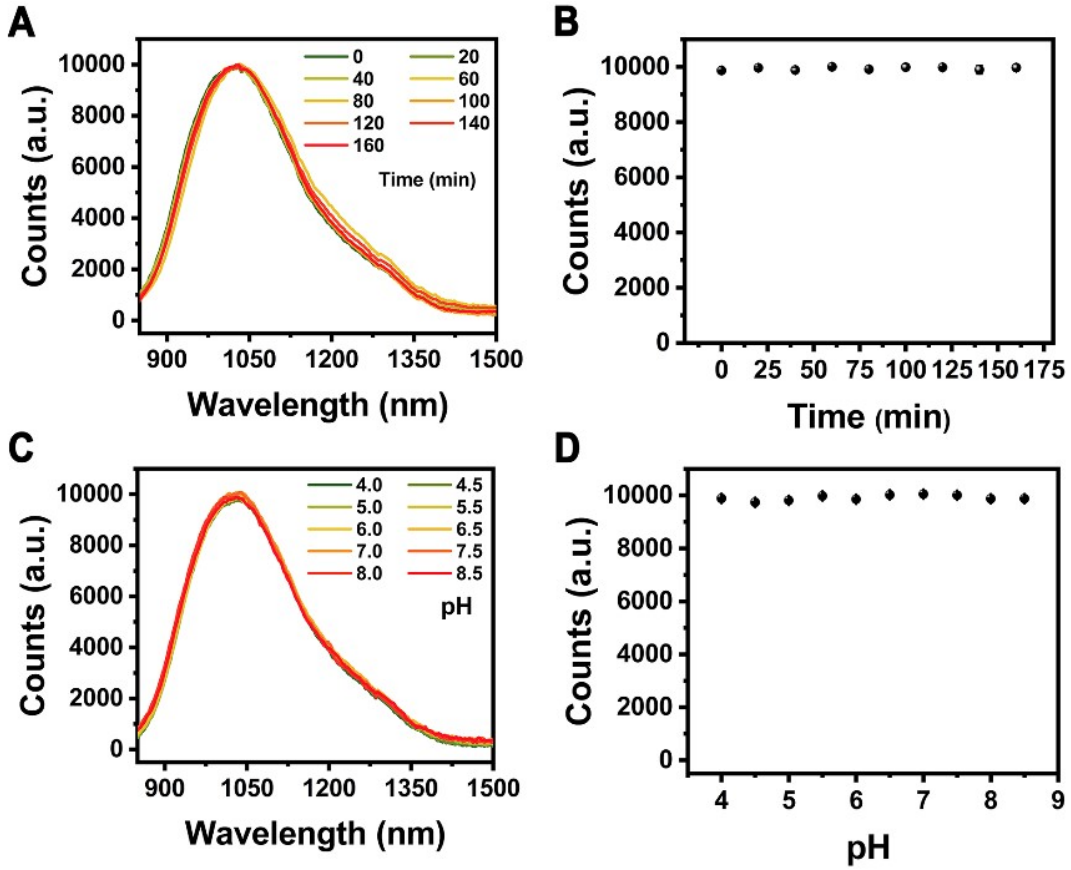


Fig. S14 (A&B) NIR-II fluorescence change of the reaction products of M-IONPs and MGO excited at different times. (C&D) NIR-II fluorescence change of the reaction of M-IONPs and MGO in different pH buffer.

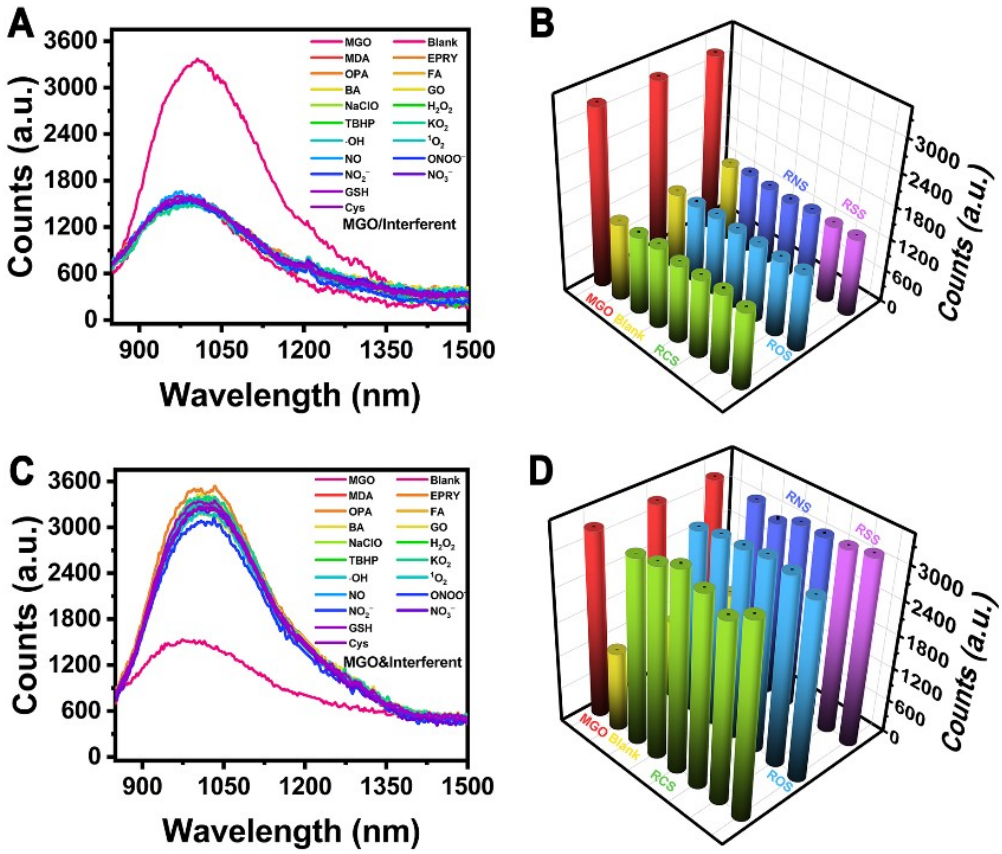


Fig. S15 (A&B) Fluorescence response of M-IONPs to 5 μM MGO or 50 μM interferences. (C&D) Fluorescence response of M-IONPs to 5 μM MGO and 50 μM interferences.

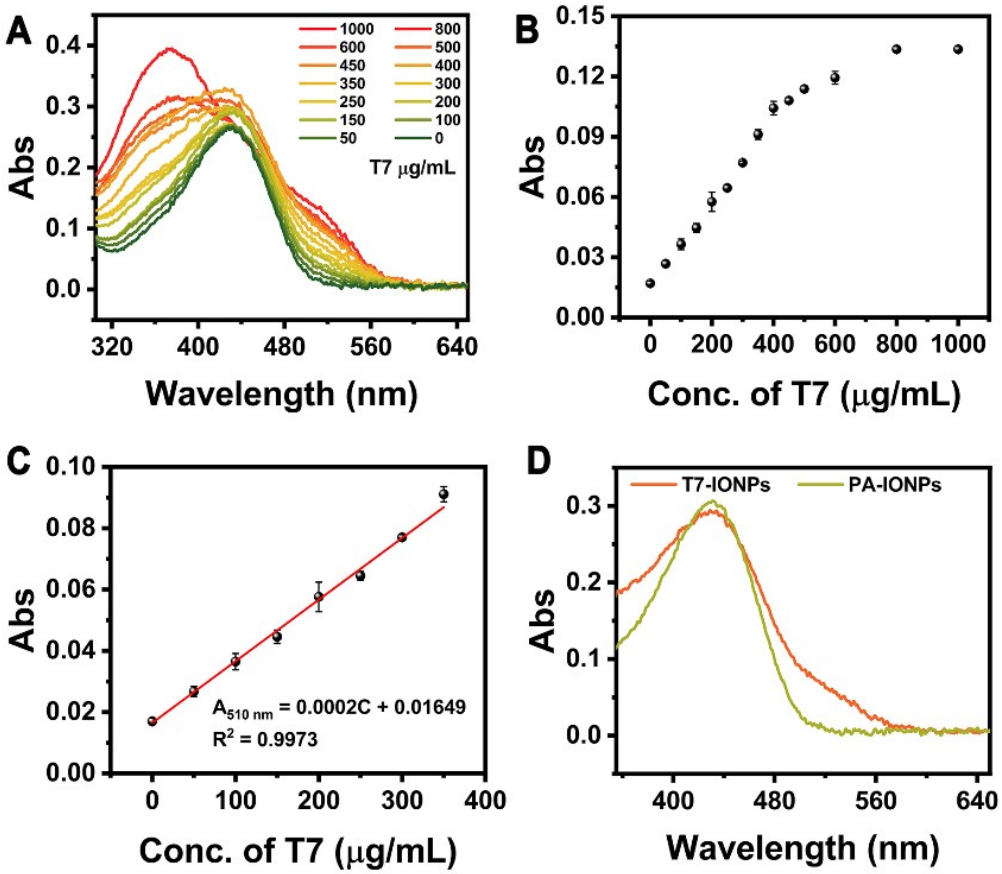


Fig. S16 (A) Absorption spectrum of different concentrations of T7 standard substance pretreated by Pauly reaction. (B&C) The relationship between absorbance and concentration of T7. (D) Absorption spectrum of T7-IONPs and PA-IONPs.

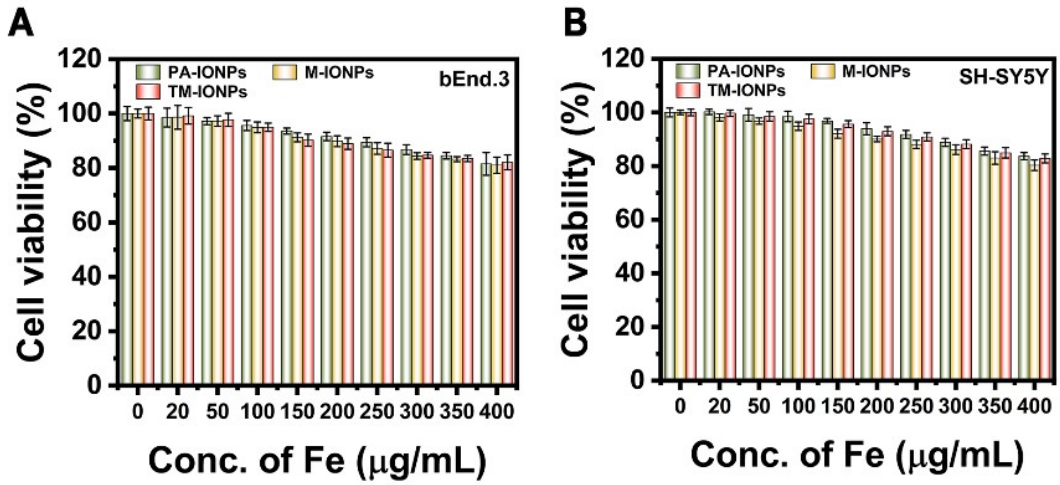
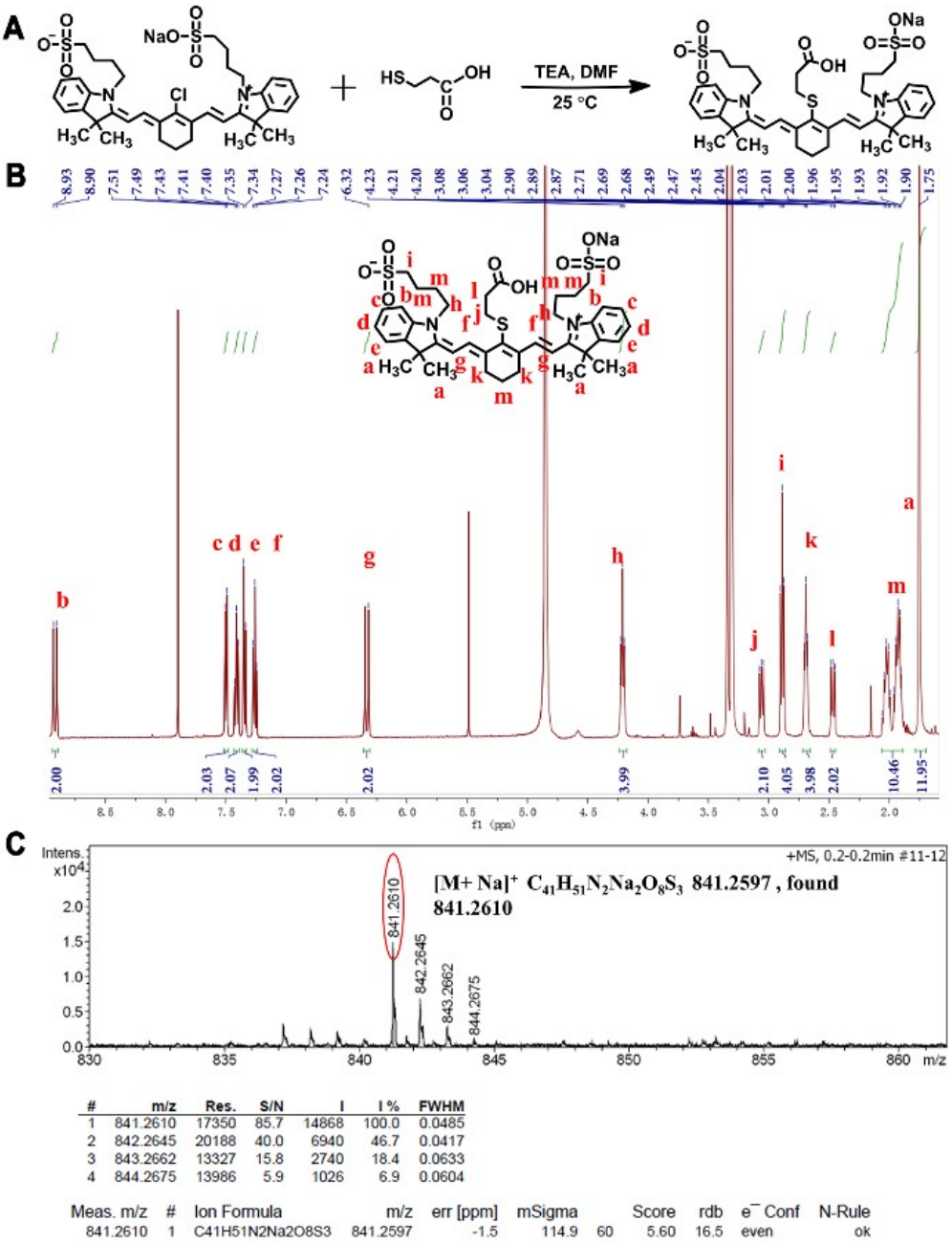


Fig. S17 Cell viability of bEnd.3 cells and SH-SY5Y cells treated with PA-IONPs, M-IONPs and TM-IONPs.



#	m/z	Res.	S/N	I	I %	FWHM
1	841.2610	17350	85.7	14868	100.0	0.0485
2	842.2645	20188	40.0	6940	46.7	0.0417
3	843.2662	13327	15.8	2740	18.4	0.0633
4	844.2675	13986	5.9	1026	6.9	0.0604

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e⁻ Conf	N-Rule
841.2610	1	C41H51N2Na2O8S3	841.2597	-1.5	114.9	60	5.60	16.5	even	ok

Fig. S18 (A) Reaction formula of carboxylated IR783. (B&C) ^{1}H NMR spectra and ESI-MS spectra of carboxylated IR783.

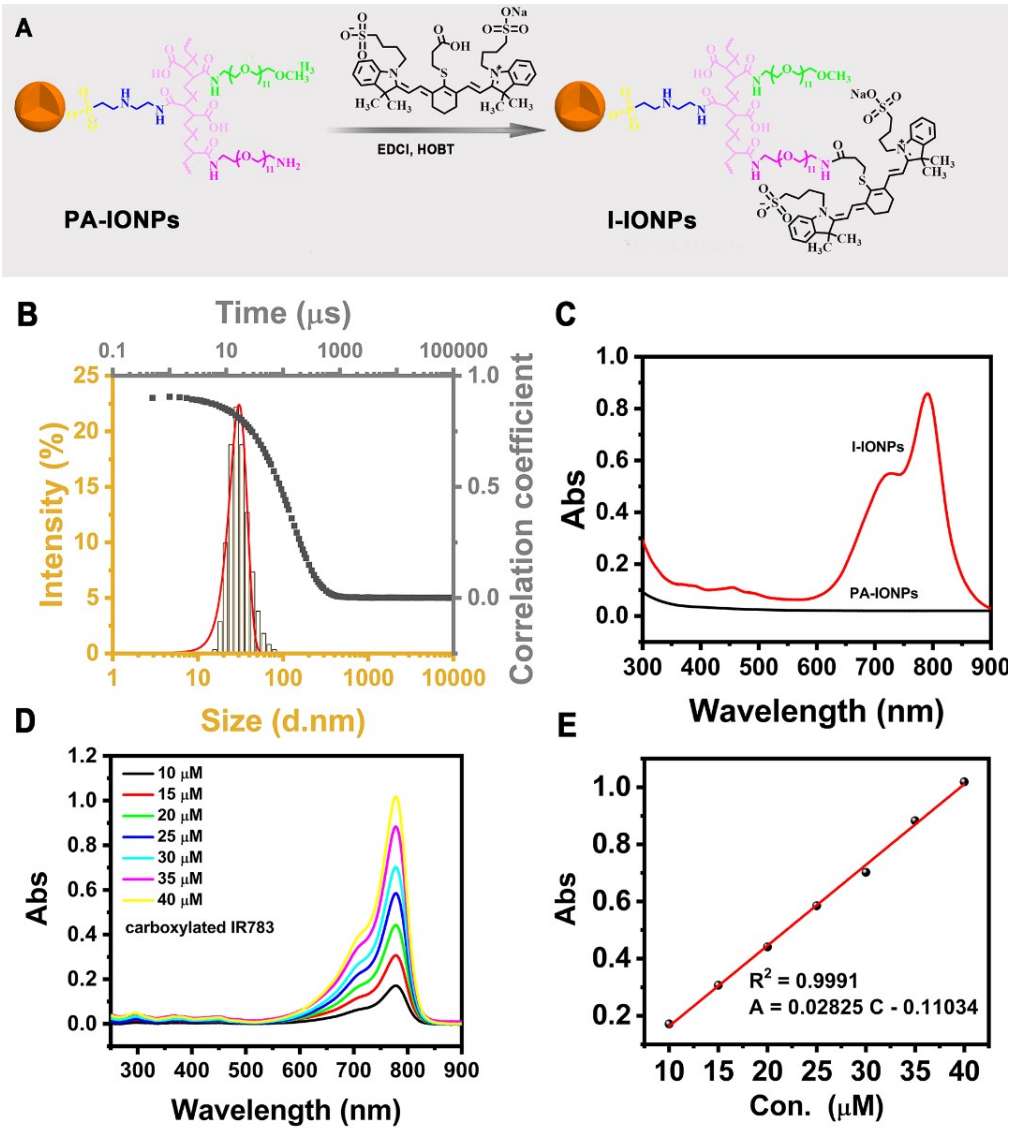


Fig. S19 (A) Schematic illustration of the preparation of I-IONPs. (B) The size distribution and intensity function of I-IONPs. (C) Absorption spectra of I-IONPs and PA-IONPs. (D&E) Absorption spectrum and calibration curve of carbxylated IR783 dye.

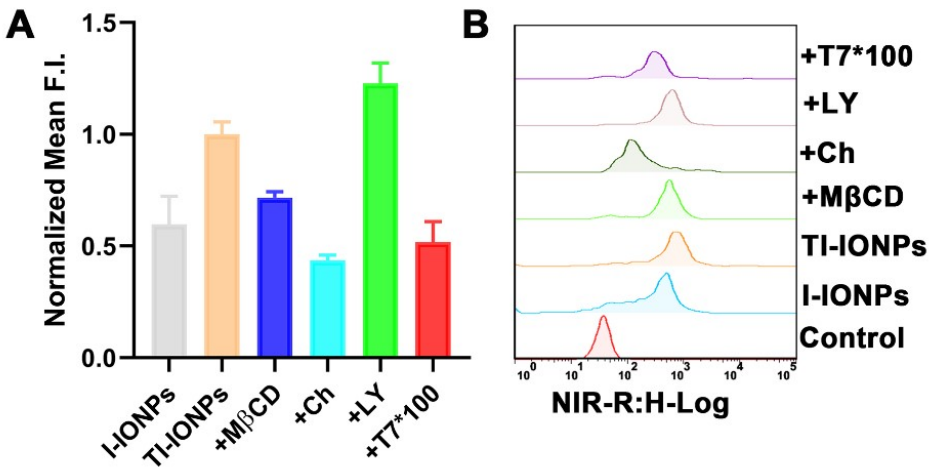


Fig. S20 (A) Mean fluorescence intensity of bEnd.3 cellular uptake of IR783 labeled nanoparticles when pretreated with different inhibitors (λ_{ex} = 714 nm, λ_{em} = 809 nm). (B) Flow cytometry analysis of cellular uptake of I-IONPs and TI-IONPs in the presence of different inhibitors pretreatment.

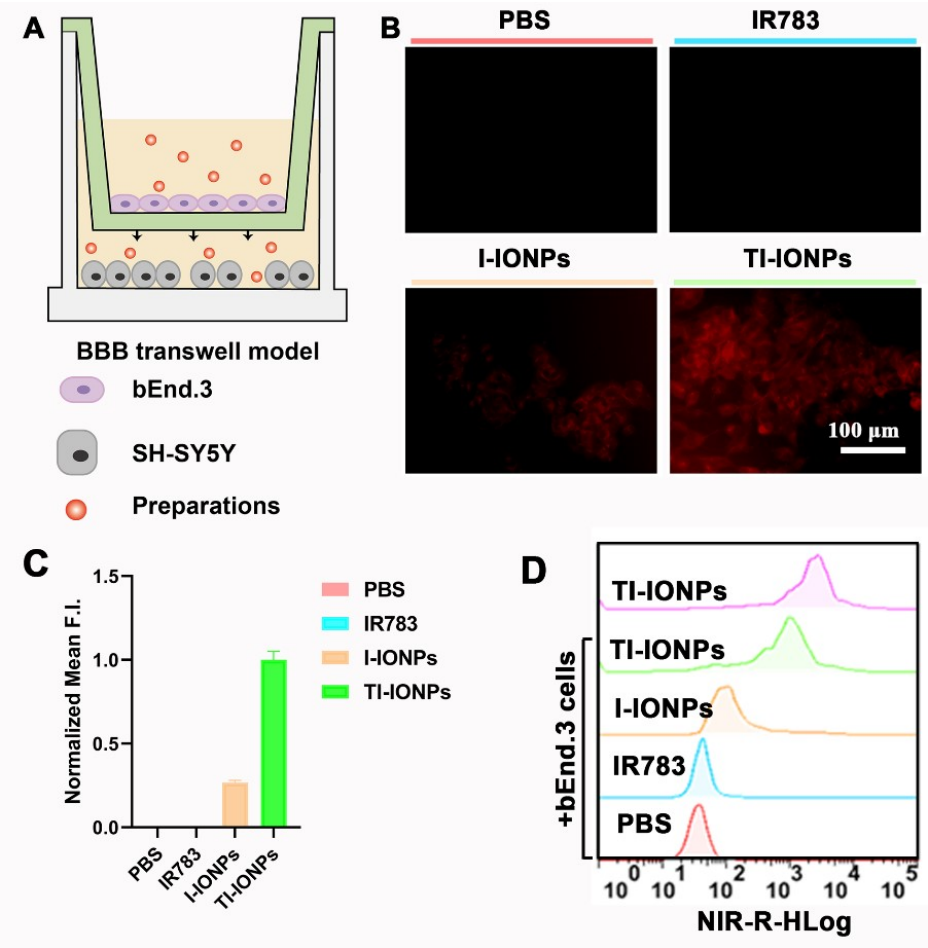


Fig. S21 (A) Fluorescence imaging showing the internalization of different formulations by SH-SY5Y cells in lower chambers of the transwell model. (B) Quantitaive analysis of normalized mean fluoresence intensity.

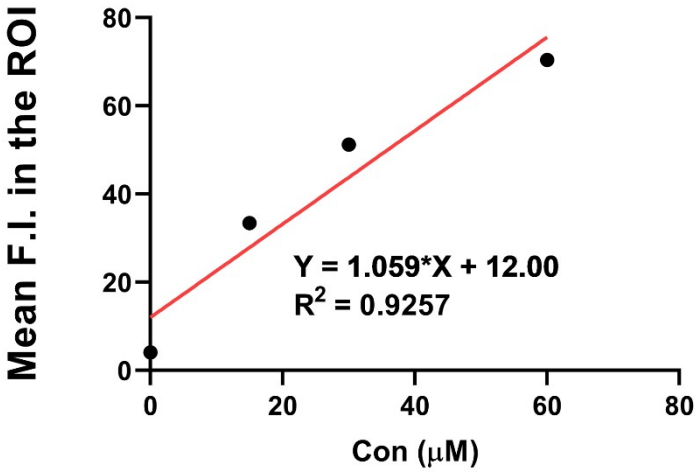


Fig. S22 Quantitative fluorescence intensity of Fig.3I. Images were analyzed as the average signal in the region of interest (ROI).

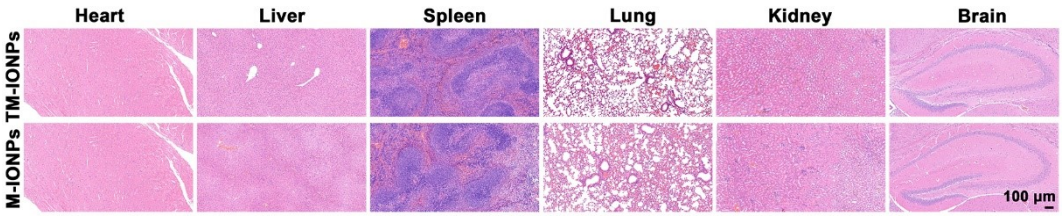


Fig. S23 H&E staining of major organs of different formulations.