Supporting Information

Iron oxide nanoparticles protected by NIR-active multidentate-polymer as multifunctional nanoprobes for NIRF/PA/MR trimodal imaging

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Experiment Section:

1.Materials and general characterization

All chemicals were used as purchased without further purification. Ultrapure water (18.25 MΩ.cm, 25° C) was used in all experiments. Transmission electronmicroscope (TEM) images were taken on a Tecnai G2 F20 S-TWIN from FEI company. Magnetization curves were obtained from lyophilized hydrophobic iron oxide NPs using a Quantum Design MPMS-XL-7 superconducting quantum interference device (SQUID) magnetometer (Quantum Design, SanDiego,CA). Density of mental ions were measured with ICP-OES Optima 7000DV purchased from PekinElmer. ^{1}HNMR spectra of the polymers were recorded on a Bruker 400MHz nuclear magnetic resonance instrument using DMSO-d_6 as the solvents. TGA curve were carried out on a SDT Q600 TGA- DSC from TA instruments. FT-IR spectra of the polymers were recorded on a Bruker Vertex 70 from Bruker Optics. UV-vis absorption spectra were taken on a PerkinElmer Lambda 25 UV-Vis absorption spectrophotometer. The particle size and zeta potential of particles were characterized on Nano-Zetasizer (Malvern Instruments) at 25℃. Photoluminescence (PL) spectra were recorded with an Edinburgh F900 fluorescent spectrometer. *In vivo* optical imaging was carried out using a Maestro GNIR Flex imaging system (CRi) along with image acquisition and analysis software from CRi. The relaxation times at low field strength were measured on a 0.55 T MRI instrument (MicroMRI, Shanghai Niumag Corp.) at 32°C. *In vivo* MRI experiments were performed on a clinical 3 Tesla horizontal bore magnet (SIEMENS, VERIO). *In vitro* and *in vivo* photoacoustic imaging were carried out using the multispectral optoacoustic tomography system(MSOT) purchased from iTheraMedical (Germany). 8-week-old Balb/c mice were obtained from Guangdong Province Laboratory Animal Center (Guangzhou, China), and maintained in the institutional animal care facility. The mice were acclimated for 1 week prior to use. All animal protocols were approved by Institutional Animal Care and Usage Committee of Shenzhen Institutes of Advanced Technology.

2. Synthesize

2.1 Synthsize of organic-soluble IONPs

Magnetic NPs were obtained via a method reported previously.[1] Typically,1g $Fe(acac)_3$ was dissolved in 15mL of diphenyl ether and 15mL of oleyamine in a 100mL three-neck round-bottom flask.The solution was dehydrated at 110°C, for 1h under N_2 atmosphere, then quickly heated to 280°C at a heating rate of 10°C/min, aged at this temperature with refluxing. After the reaction, the solution was allowed to cool down to room temperature. The IONPs were extracted upon the addition of 50mL ethanol, followed by centrifuging with rotating speed 10000rpm for 10min.The IONPs were weighted and dispersed in chloroform with a concentration 20mg/mL.

2.2 Synthesize of NIR-emitting multidentate polymers (820-PIMA-Dopa)

In a 20 mL screw thread vial equipped with a magnetic stirring bar, 100mg poly (isobutylene-alt-maleic anhydride) (PIMA,Mw~6000g/mol ,~0.66mmol monomer units) with 200mg 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride（EDC•HCl,1.04mmol） and 66mg N-Hydroxysuccinimide(NHS,0.57mmol) were dissolved in 5mL DMSO, then add 100uL triethylamine. The solution was stirring for 1h at room temperature,then 63.5mg dopamine hydrochloride(0.33mmol) and 25.5mg cysteamine(0.33mmol) were added. The solution was pale yellow and heated to 80°C in oil bath, stirred overnight. Then 20mg IR 820 was added in this solution,decreased the temperature to 70°C and reacted in dark over night. The obtained mixture was dialyzed against ultrapure water for two days(membrane cutoff 7000D). The dialyzed water solution was freeze-dried and obtained blue powder.

2.3 Ligand exchange of organic IONPs nanoparticles

To 10mg 820-PIMA-Dopa powder was added 100uL oleyamine-capped iron oxide particles (20mg/mL in chloroform) in a 1.5mLcentrifuge tube. Then added 1mL chloroform and 100uL Tetramethylammonium Hydroxide (TMAOH, 25% aqueous solution). The mixture was slightly shaked and quickly the dark supernatant appeared. Acquired the supernatant and discarded the organic phase solution.The supernatant hydrophilic nanoparticles were washed with acetone and centrifuged, acheved dark pellet and re-dissolved in ultrapure water, followed by centrifuging, discard the residual precipitate and acquired clear solution of aqueous IONPs. Excess free ligands were removed by applying 3-4 rounds of concentration/ultrapure water using a centrifugal filtration device (Millipore, Mw cutoff=50kDa). At last, hydrophilic 820-PIMA-Dopa polymer capped iron oxide particles were dissolved in water or buffer (PBS, pH7.4) to the concentration 10mM Fe ions measured by ICP-OES.

2.4 Structrual characterization

^{1}H NMR spectra was recorded on a Bruker 400MHz nuclear magnetic resonance instrument using DMSO-d_6 as the solvents. TEM images were acquired with FEI Tecnai G2 F20 S-TWIN. UV-vis absorption spectra were taken on a PerkinElmer Lambda 25 with wavelength range from 350nm to 800nm. Hydrophilic IONPs in aqueous solution were lyophilized to acquired solid pellet for analysis. FTIR spectra was recorded using a Fourier transform infrared spectrometer, Bruker Vertex 70 with wavenumber 0 to 4000cm^{-1}. TGA curve was measured with SDT Q600 from room temperature to 600°C under N_2 atmosphere. Magnetization curves were obtained using a Quantum Design MPMS-XL-7 superconducting quantum interference device (SQUID) magnetometer in a 2T magnetic field intensity. Inductively coupled plasma optimal emission spectrometry (ICP-OES)

was used to measure the contents of the iron ions. The relaxation times at low field strength were measured on a 0.55 T MRI instrument.

2.5 Colloidol stability tests

Phosphate buffer(pH7.4) was adjusted to different pH range from 4 to 10 with HCl or NaOH solution, measured with pH meter. 351mg NaCl was dissolved in 3mL ultra-pure water to concentration 2M, then 2mL solution was taken to diluted to 0.5,1M. As-prepared polymer coated iron oxide NPs (10mM Fe^{3+}) with 100uL volume were diluted in 900uL different buffer solution:pH4 pH7, pH10 in PBS and 0,0.5,1,2M NaCl in water. All samples were placed in 2mL sample vials. The photos of these samples were acquired in different buffer for 20 days to test polymer coated IONPs colloidal storage stability.

2.6 Dynamic light scattering(DLS) measurements

As-*p*repared 820-PIMA-Dopa coated iron oxide NPs (10mM Fe^{3+}) 100uL were diluted in 900uL different buffer: pH 4-10 phosphate buffer and 0, 0.5, 1, 2 M NaCl solution. All samples' hydrodynamic sizes were measured using Nano-Zetasizer (Malvern Instruments) at 25^{o}C for 3 times. The sample in water only was used to measure zeta potential and particle storage stability for 20 days.

2.7 Fluorescence stability compared with free IR 820

820-PIMA-Dopa coated iron oxide NPs samples diluted in PBS(pH4-10) and NaCl (0-2M) were measured fluorescence spectra on a Fluorescence Lifetime and Steady State Spectroscopy(FS920). With excitation wavelength 680nm and scan slit 4nm, scanned from 700nm to 900nm, particles showed a strong fluorescence emission at 762nm. After 3 times measurement of each sample, record intensity of maximum emission wavelength at 762nm and drawing graph of fluorescence changed with the variation of pH values and NaCl concentrations. As control, 1mg IR-820 was weighted and diluted to 10ug/mL by water, with excitation wavelength 740nm and scan slit 2nm,scanned from 760nm to 900nm, we obviously find a strong emission at 812nm. IR -820 was diluted in different buffer same as iron oxide NPs to 10ug/mL. Fluorescence spectra were measured as mentioned and record intensity of wavelength 812nm to draw graph of free IR- 820 fluorescence changed with the variation of pH values and NaCl concentrations. Also, the photos of the samples IR-820 in different buffer were taken. The photostability experiment of the free IR-820 and 820-PIMA-Dopa coated iron oxide NPs samples in water were continuously irradiated for 50 minutes by a xenon lamp (740nm, slit 8nm). Samples in water were also used to measure fluorescence storage stability for 20 days.

2.8 Optical imaging of hydrophilic NIR NPs in vitro

Hydrophilic NIR NPs (10mM) in water and oleyamine capped iron oxide NPs (500uL) in chloroform were each placed in two sample vials (2mL). Chloroform and water were added to form a biphasic system: chloroform/water. A magnet was placed between this two vials. Optical imaging was carried out using a Maestro GNIR Flex imaging system (CRi) along with image acquisition and analysis software from CRi. When the tunable liquid crystal filter in the Nuance GNIR CCD camera (Cambridge Research & Instrumentation Inc., CRi) was stepped by an increment of 10 nm from 650 to 950 nm. The deep red excitation was performed for imaging. The same procedure was carried out on the samples for stability test.

2.9 In vitro cytotoxicity assay with CCK-8

We assessed the cytotoxicity of the 820-PIMA-Dopa coated iron oxide NPs using CCK-8 (Cell Counting Kit-8) assay on MCF-7 cell (human breast adenocarcinoma cell line).The

lyophilized iron oxide NPs of different concentrations (0, 1, 10, 50, 100, 200ug/mL) were tested. The CCK-8 assay is a colorimetric test based on the cellular reduction of WST-8 by the mitochondrial dehydrogenase of viable cells, forming a orange formazan which can dissolved in aqueous solution and measured spectrophotometrically. CCK-8 solution was purchased from Dojindo laboratories(Japan). Cells were first seeded into 96-well microplates (5000cell/100 μL/well), and the plates were placed in an incubator overnight to allow adherence. After 24 hours, the nanoparticles were applied directly to the wells using a multichannel pipette, and the cultures were incubated for 24 h at 37°C. After incubation,10uL CCK-8 solution was added to each well and continued incubate for 4hours at 37°C.The absorbance at 450 nm was measured using the Thermo Scientific Multiskan GO microplate reader. The cell viability obtained from the absorbance measurements was expressed as a fraction of viable cells and normalized to that of cells that were not exposed to reagents.

2.10 Multimodol imaging of axillary lymph node in vivo

Optical Imaging: IONPs@820-PIMA-Dopa in water (1mM,100uL) was subcutaneously injected in the front right paw of a 8-week-old balb/c mice. After 24hours, hair of the right limb were removed and anesthesia, and the mice was placed in the Maestro GNIR Flex imaging system. The same measurement was applied to acquired optical imaging of axillary lymph node *in vivo*. MR and photoacoustic imaging were also measured with the same mice.

MR Imaging: MRI experiments were performed on a clinical 3 Tesla horizontal bore magnet (SIEMENS, VERIO).The pulse sequence time parameters (TE/TR) of T2 weighted imaging were as follows: TE = 51 ms, TR = 5000 ms.

Photoacoustic Imaging: Photoacoustic imaging generation capabilities of 820-PIMA-Dopa coated iron oxide NPs was studied using cylindrical tissue mimicking phantom. Two inclusions having 4 mm diameter were created within the phantom to evaluate the photoacoustic signal generation from IONPs@820-PIMA-Dopa. IONPs@820-PIMA-Dopa in water and blank saline were placed separately into the phantom inside the imaging chamber of the MOST (MultiSpectral Optoacoustic Tomography) machine. Photoacoustic signals were measured by illuminating from 680nm to 900nm in 5nm steps. The animal injected with IONPs@820- PIMA-Dopa was placed in prostrate position inside the imaging chamber, with nanosecond pulsed laser excited at 900nm.

References:

1. Z. Xu, C. Shen, Y. Hou, H. Gao and S. Sun, *Chem. Mater.*, 2009, 21, 1778-1780

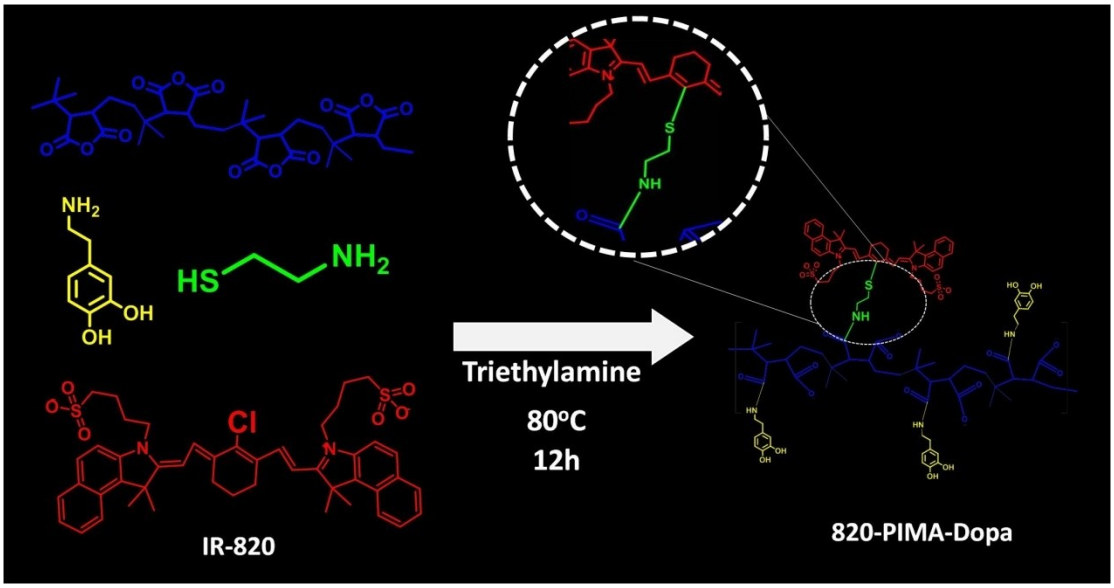

Figure S1. Schematic illustration of the synthesis of the near-infrared catechol-based multidentate polymers 820-PIMA-Dopa: the NIR-emitting multidentate polymers (820-PIMA-Dopa) were produced using poly (isobutylene-alt-maleic anhydride)(PIMA) as backbone, dopamine as coordinating moieties, and Indocyanine Green analogues (IR-820) were linked through cysteamine as NIR optical-active moieties.

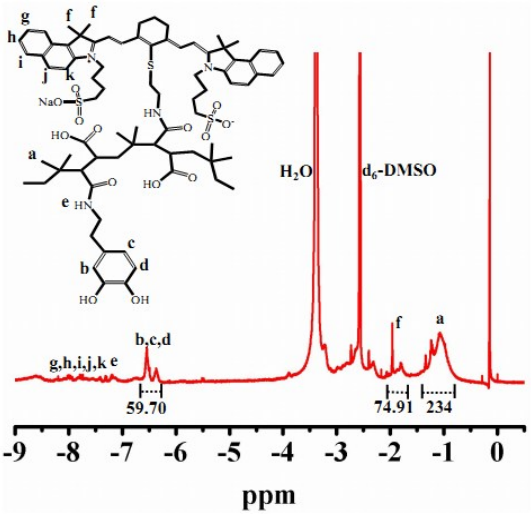

Figure S2. a) ^{1}H NMR spectrum of 820-PIMA-Dopa.

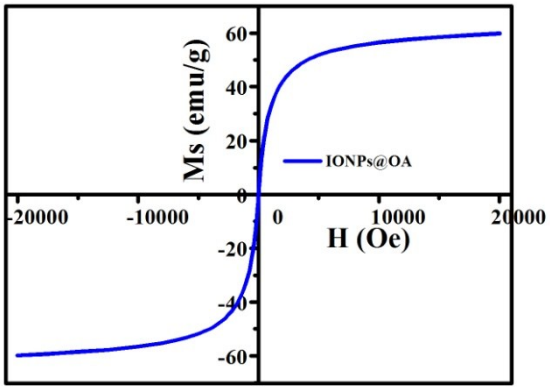

Figure S3. Magnetic hysteresis loop of IONPs@OA at 300K.

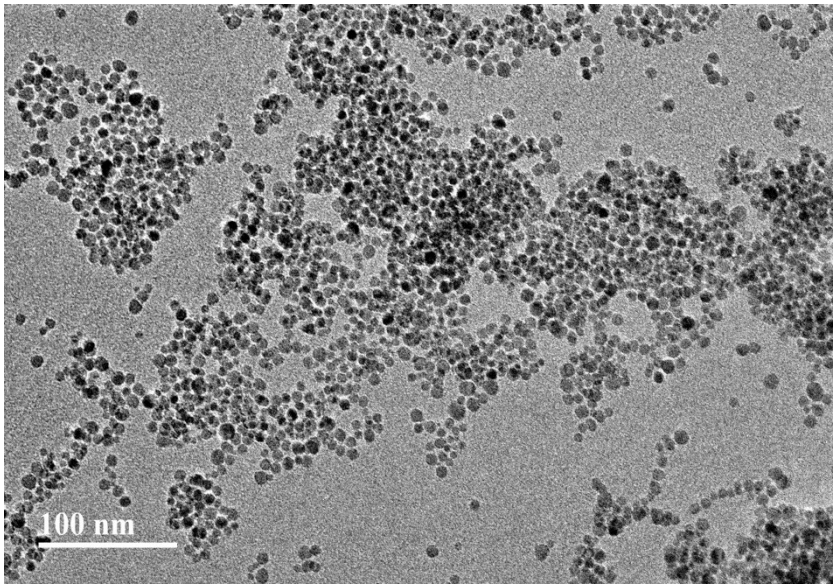

Figure S4. TEM images of IONPs@OA.

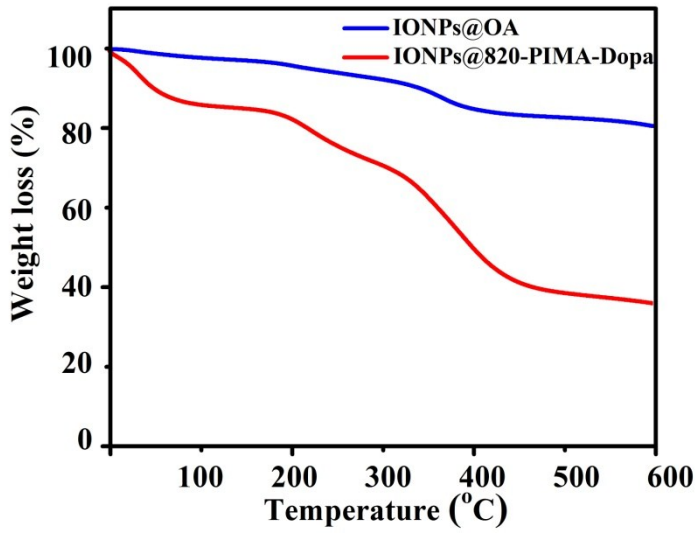

Figure S5. TGA analysis of IONPs@OA and IONPs@820-PIMA-Dopa

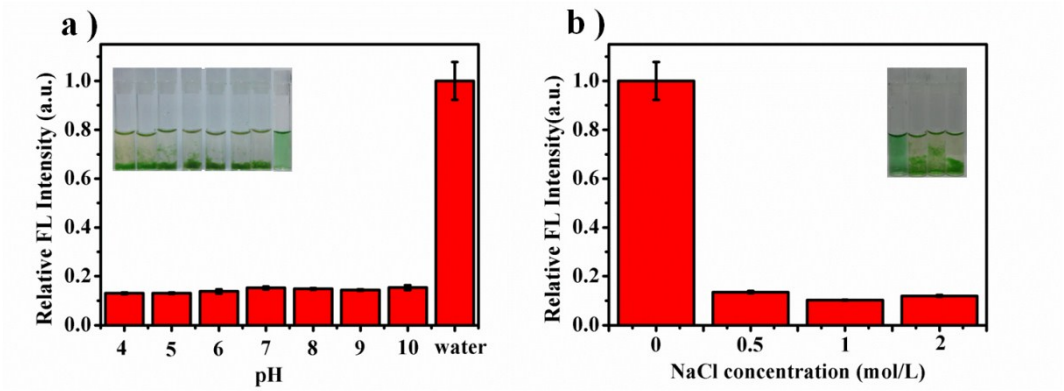

Figure S6. Fluorescent stability of IR 820 in buffer solutions with different pH (a) and different ion concentration (b).

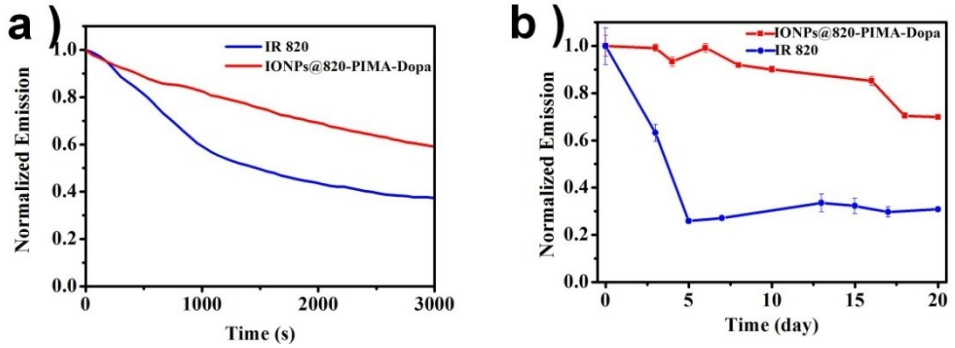

Figure S7. Photo-bleaching and long term storage Fluorescent stability of IR 820 and IONPs@820-PIMA-Dopa.

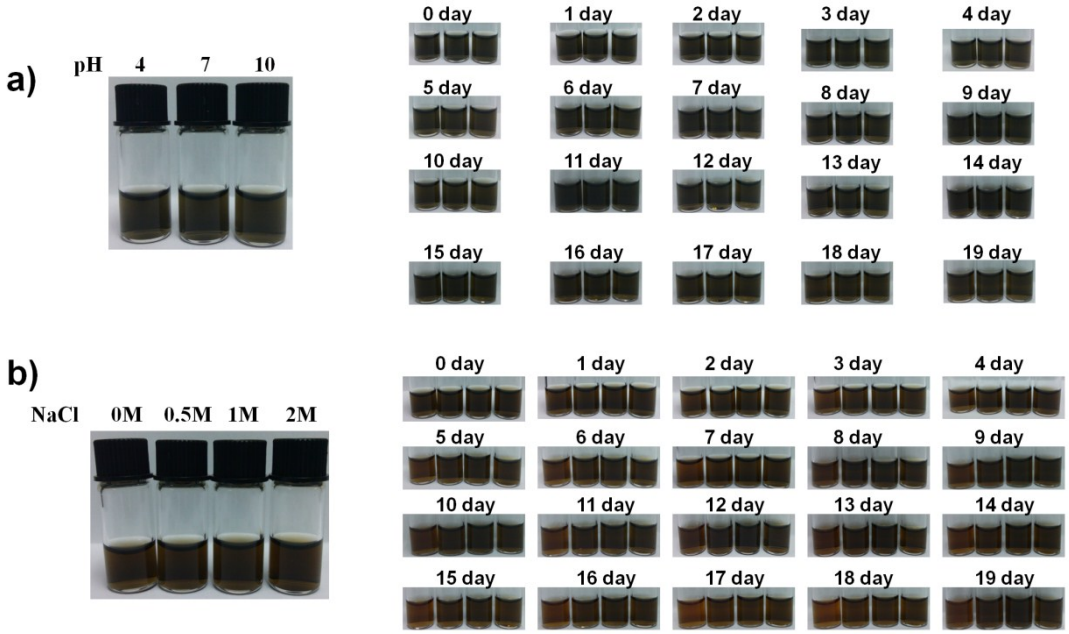

Figure S8. Long-term storage experiments of IONPs@820-PIMA-Dopa in different pH (a) and ions concentration (b).

	Quantum Yield
ICG in DMSO	13%(as standard)
IR 820 in water	0.783%
IONPs@820-PIMA-Dopa in water	1.196%

Table S1. The quantum yield measurement results.