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Supporting Information

Polymeric engineering of AIEgens for NIR-II fluorescence imaging and detection of abdominal metastases of ovarian cancer in vivo

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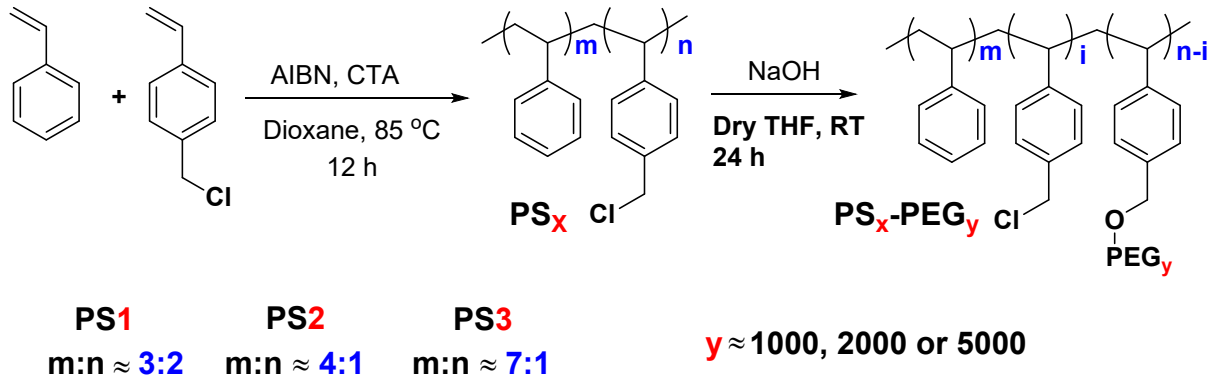
Instruments and materials

The ^{1}H NMR characterization were measured at room temperature using Bruker Ultra Shield Plus 400 MHz NMR instrument. UV-Vis absorption spectra were recorded on Lambda 750 (PerkinElmer, America). Photoluminescent spectra were measured using a QM40 (PTI, America) system with a xenon lamp and 730 nm laser as the excitation source. Transmission electron microscopy (TEM) was conducted on a JEOL transmission electron microscope (JEM-2100) at an acceleration voltage of 100 kV. The number-average molecular weight (*M*n) and weight-average molecular weight (*M*w) of the polymers were characterized in THF by gel permeation chromatography at 35 °C (polystyrene as standard). Dynamic light scattering (DLS) measurements were taken using a Malvern nanoparticle size zeta potential analyzer. The NIR luminescence images were collected with an InGaAs-based NIR camera under the excitation of external 730 nm laser.

All chemicals were purchased from commercial sources and used without further purification. All solvents were purified before use. The TPA-BBTD was synthesized according to the previous reports.

Synthesis of PS-PEG



Scheme S1. Synthetic routes of PS-PEG with different PS and PEG content.

PS1-3: PS1-3 was synthesized using RAFT of styrene and 4-(chloromethyl)styrene. Typically, styrene (PS1: 0.69g, 6.55 mmol, PS2: 1.29 g, 12.5 mmol, PS3: 2.00 g, 19.2 mmol), 4-

(chloromethyl) styrene (PS1: 0.5 g, 3.26 mmol, PS2: 0.375 g, 2.5 mmol, PS3: 0.36 g, 2.4 mmol), chain transfer agent 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (0.056 g, 0.20 mmol), and initiator AIBN (0.008 g, 0.04 mmol) were mixed in a 25 mL Schlenk flask, and the solution was degassed by three freeze-pump-thaw cycles. The mixture was then heated under argon at 85 ^{o}C for 24 h. The polymerization was stopped by cooling the reaction flask in liquid nitrogen. The resulting copolymer was isolated as a pink powder after dissolving in DCM and precipitating into 40-fold MeOH for three times.

PS1- Content of Ph-CH_2Cl (~35%) ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.08 (m, 3.48 H), 6.55 (m, 2.59H), 4.53 (bs, 1H), 1.25-2.05 (m, 3.72H);

GPC (THF, polystyrene standard): M_w 6300 g/mol, M_n 5900 g/mol, PDI 1.07. m: ~32, n: ~17.

PS2- Content of Ph-CH_2Cl (~20%) ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.13 (m, 7.10 H), 6.55 (m, 5.09H), 4.55 (bs, 1.00H), 1.25-2.15 (m, 7.66H);

GPC (THF, polystyrene standard): M_w 6500 g/mol, M_n 6100 g/mol, PDI 1.07. m: ~44, n: ~11.

PS3-Content of Ph-CH_2Cl (~12.5%) ^{1}H NMR (400 MHz, $CDCl_3$, δ) 7.13 (m, 12.26 H), 6.56 (m, 7.38H), 4.56 (bs, 1H), 1.25-2.55 (m, 12.43H);

GPC (THF, polystyrene standard): M_w 6100 g/mol, M_n 5700 g/mol, PDI 1.06. m: ~46, n: ~7.

PS-PEG: PS1, PS2 or PS3, mPEG-OH (Mn~1000, 2000 or 5000) (two equivalent to chloromethyl groups on the PS copolymer), and NaOH were added into anhydrous THF (1.5 mL). The mixture was stirred for 48 h at room temperature under argon. The reaction mixture was filtered to remove the generated salt and residual base, and the filtrate was evaporated to dryness. The crude graft polymer was then dialyzed against DI water for three days and lyophilized to give a white powder.

PS1-PEG1000: GPC (THF, polystyrene standard): M_w 19900 g/mol, M_n 17300 g/mol, PDI 1.14. The number of PEGylated units was calculated to be ~11.

PS2-PEG1000: GPC (THF, polystyrene standard): M_w 16500 g/mol, M_n 15100 g/mol, PDI 1.10. The number of PEGylated units was calculated to be ~9.

PS3-PEG1000: GPC (THF, polystyrene standard): M_w 13700 g/mol, M_n 12100 g/mol, PDI 1.13. The number of PEGylated units was calculated to be ~6.

PS1-PEG2000: GPC (THF, polystyrene standard): M_w 24100 g/mol, M_n 20100 g/mol, PDI 1.20. The number of PEGylated units was calculated to be ~7.

PS2-PEG2000: GPC (THF, polystyrene standard): M_w 20000 g/mol, M_n 17100 g/mol, PDI 1.17. The number of PEGylated units was calculated to be ~5

PS3-PEG2000: GPC (THF, polystyrene standard): M_w 15800 g/mol, M_n 13600 g/mol, PDI 1.16. The number of PEGylated units was calculated to be ~5.

PS1-PEG5000: GPC (THF, polystyrene standard): M_w 32700 g/mol, M_n 24300 g/mol, PDI 1.30. The number of PEGylated units was calculated to be ~4.

PS2-PEG5000: GPC (THF, polystyrene standard): M_w 27300 g/mol, M_n 22200 g/mol, PDI 1.23. The number of PEGylated units was calculated to be ~3.

PS3-PEG5000: GPC (THF, polystyrene standard): M_w 23100 g/mol, M_n 19100 g/mol, PDI 1.01. The number of PEGylated units was calculated to be ~3.

Preparation of NIR-II AIEgens-doped nanoparticles by using different PS-PEG

A mixture of TPA-BBTD (1 mg), PS-PEG (2 mg) and THF (2 mL) was sonicated to obtain a clear solution. The mixture was quickly injected into DI water (20 mL). The mixture was stirred in fume hood for 2 h and concentrated under vacuum. Then, the solution was filtered through a membrane filter (diameter = 0.22 μm) to obtain the NIR-II AIEgens-doped nanoparticles . The NIR-II AIEgens-doped nanoparticles were stored in 4 ^{o}C for further usage.

Calculation of the dye contents in organic nanoparticles

A certain volume of aqueous solution contained organic nanoparticles were dried. The weight of the nanoparticles powder was measured for mass concentration calculation, termed C_{NPs}. The nanoparticles powder was then dissolved in a certain volume of tetrahydrofuran (THF), and BBTD were extracted in THF. The absorbance of BBTD at 760 nm in THF was measured. According to the extinction coefficient of BBTD in THF at 760 nm (1.5×10^4 $M^{-1}cm^{-1}$), the

molar concentration of BBTD ($C_{dye\text{-}molar}$) in THF could be obtained. Then the dye content of different organic nanoparticles can be calculated by following equation:

$$Dye\ content = \frac{m_{dye}}{m_{NPs}} = \frac{M_{dye}C_{dye-molar}}{C_{NPs-mass}} \quad (1)$$

Where m_{dye} is the mass of BBTD in THF solution; m_{NPs} is the mass of the organic nanoparticles in THF solution; M_{dye} is the molar mass of BBTD; $C_{dye\text{-}molar}$ is the molar concentration of BBTD in THF solution; $C_{NPs\text{-}mass}$ is the mass concentration of organic nanoparticles in THF solution (according to the C_{NPs}).

Fluorescence quantum yield measurement

To measure the quantum yield of the NIR-II nanodots, the reference fluorophore is IR26 dissolved in DCE (QY = 0.5%), Ex = 730 nm. The quantum yield was calculated in the following manner.

$$\phi = \phi_{ref} \times \left(n_{sample}^2/n_{ref}^2\right)\left(I_{sample}/A_{sample}\right)\left(A_{ref}/I_{ref}\right) \quad (2)$$

Difference concentrations at or below OD 0.1 were measured and the integrated fluorescence was plotted against absorbance for every fluorescent molecular. Comparison of the slopes led to the determination of the quantum yield of fluorophore.

Cell culture

Human serous ovarian cancer cell line A2780 and SKOV3, and human stomach adenocarcinoma cell line Ags, were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The Ags cells were grown in DMEM with 10% FBS (fetal bovine serum) at 37 ºC and 5% CO_2. The A2780 cells were grown in RMPI 1640 with 10% FBS (fetal bovine serum) at 37 ºC and 5% CO_2. The SKOV3 were grown in McCOY5A supplemented with 10% FBS at 37 ºC and 5% CO_2. All cells were planted on 14 mm glass coverslips and keep to adhere for 24 h.

Cytotoxicity test

The *in vitro* cytotoxicity was measured using a standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay in Ags cell lines. Briefly, cells growing in log phase were seeded into 96-well cell culture plate at the number of 1×10^4/well. NIR-AIEdots-1~3 were added into the wells at the TPA-BBTD concentration of 0, 0.01, 0.02, 0.05, 0.1, 0.2 mg/mL. For the negative control group, 1 μL/well solvent was diluted in DMEM with the final concentration of 1 %. The cells were incubated for 24 h at 37 °C under 5 % CO_2. The combined MTT/PBS solution was added to each well of the 96-well assay plate and incubated for an additional 4 h. After the removing of culture solution, 200 μL DMSO was added into each well, shaking for 10 min at shaking table. An enzyme-linked immunosorbent assay (ELISA) reader was used to measure the OD570 (absorbance value) of each well. The following formula was used to calculate the viability of cells:

Viability (%) = (mean of absorbance value of treatment group / mean of absorbance value of control) × 100

Tumor Xenografts

Animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee. Animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee, Laboratory Animal Center, Nantong University. Tumor cells were harvested when they reached near confluence by incubation with 0.05% trypsin-EDTA. Cells were pelleted by centrifugation and resuspended in sterile PBS. For subcutaneous tumor model, the SKOV3 cells (5×10^6 cells/site) were implanted subcutaneously into the four-week-old female athymic nude mice. When the tumors reached 0.5 cm in diameter (three weeks after implantation), the tumor-bearing mice were subjected to imaging studies. For peritoneal metastases tumor model, the A2780 cells (1×10^7 cells/site) were implanted intraperitoneally into the four-week-old female athymic nude mice. After 6-8 weeks, the tumor-bearing mice were subjected to imaging studies.

Fluorescence imaging in vitro and in vivo

The *in vivo* and *in vitro* fluorescence imaging were performed by using the homemade imaging system fabricated by ourselves. Animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee, Laboratory Animal Center, Nantong University. The luminescent signals were collected using an InGaAs camera. The system was equipped with different long-pass filters (1000LP, 1100LP, 1200LP, 1300LP and 1400LP). The excitation source for *in vitro* and *in vivo* experiments was a 730 nm laser. The fluorescence images was analyzed by the Bruker imaging software.

Histological analysis

In the test group, nude mice (n = 3) were intravenously injected with NIR-II AIEdots-1~3 at a total dose of 1 mg/mL (200 μL). And nude mice (n = 3) with no injection were selected as the control group. Tissues were harvested from test and control groups after 24 h and 1 week intravenous injection. The heart, liver, spleen, lung and kidney were removed, and fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The sections were observed under an optical microscope. After fluorescence imaging *in vivo*, the tissues were harvested and fixed in 4% paraformaldehyde for H&E staining analysis, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The sections were observed under an optical microscope.

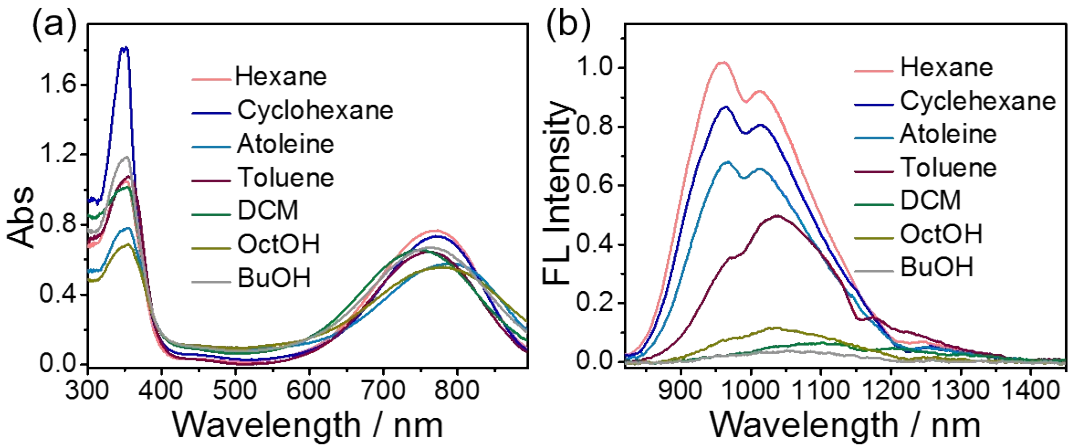
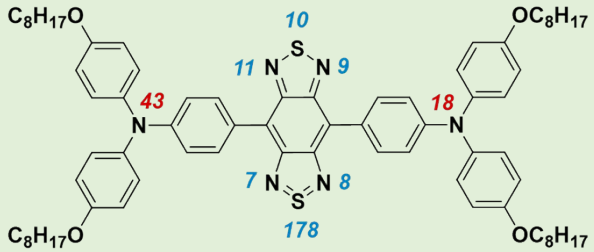


Figure S1. Absorption (a) and emission (b) spectra of TPA-BBTD in different solvents.

Fluorophores



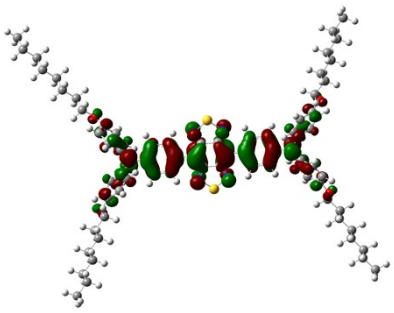
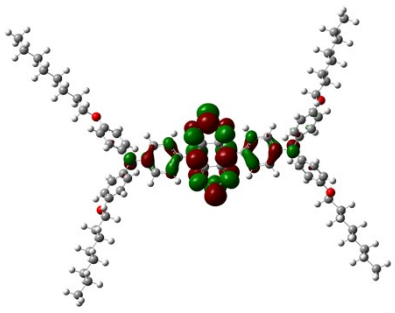
		HOMO	LUMO
HOMO		**LUMO**	
			
Electron cloud density of labeled atoms			
	7(N)	2.28%	8.25%
	11(N)	2.27%	8.28%
	8(N)	2.09%	8.44%
	9(N)	2.09%	8.46%
	10(S)	0.37%	11.26%
	178(S)	0.37%	11.25%
	Total	***9.47%***	***55.94%***
	43(N)	7.53%	1.49%
	18(N)	6.82%	1.73 %
	Total	***14.35%***	***3.22%***

Figure S2. Calculated orbital distribution of **TPA-BBTD**

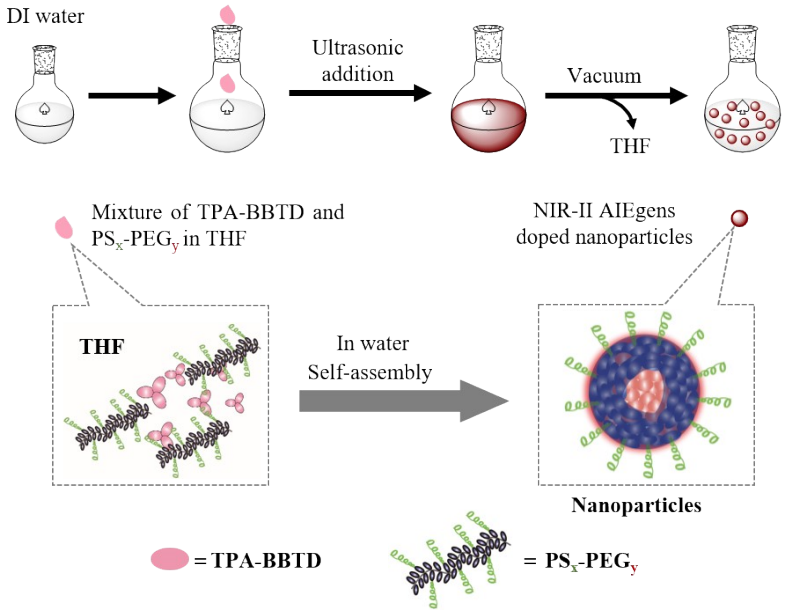


Figure S3. Schematic illustration of the preparation with NIR-II AIEgens-doped nanoparticles by using different PS-PEG as loading matrix.

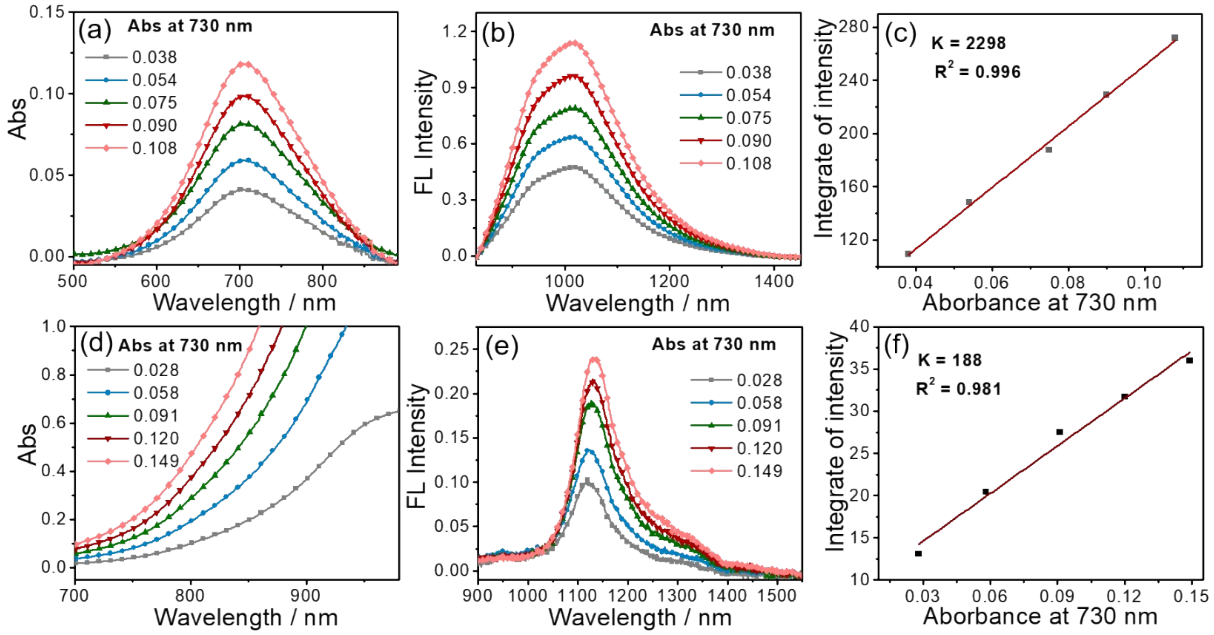


Figure S4. Fluorescence quantum yield measurement of the nanodot composed by TPA-BBTD and PS3-PEG2000 (NIR-II AIEdots-2) in aqueous solution. Absorption spectra of different concentrations of the nanodot in aqueous solution and the reference compound (IR26, in 1, 2-dichloroethane, QY = 0.5%, d). Corresponding fluorescence spectra of different concentrations of NIR-II AIEdots-2 (b) and IR26 (e), respectively. Excitation: 730 nm. (c) Integrated fluorescence intensity plotted as a function of absorbance at 730 nm for the NIR-II AIEdots-2 solutions based on the measurements in a) and b). The data was fitted into a linear function with a slope of 2298. (f) Integrated fluorescence intensity plotted as a function of absorbance at 730 nm for IR26 based on the measurements in d) and e). The data was fitted into a linear function with a slope of 188.

PS_x-PEG_y	NIR-II AIEgens loading ratio	QY	Diameter	PDI
PS1-PEG1000	21.4%	4.1%	43 nm	0.213
PS2-PEG1000	24.5%	4.3%	50 nm	0.174
PS3-PEG1000	29.0%	5.5%	15 nm	0.143
PS1-PEG2000	23.1%	4.5%	54 nm	0.181
PS2-PEG2000	22.0%	4.8%	43 nm	0.135
PS3-PEG2000	26.3%	5.4%	20 nm	0.128
PS1-PEG5000	16.1%	3.9%	42 nm	0.198
PS2-PEG5000	14.2%	4.6%	35 nm	0.246
PS3-PEG5000	19.2%	5.1%	30 nm	0.171

Table S1. Characterizations of the nanoparticles that consisted by BBTD and different PS-PEG.

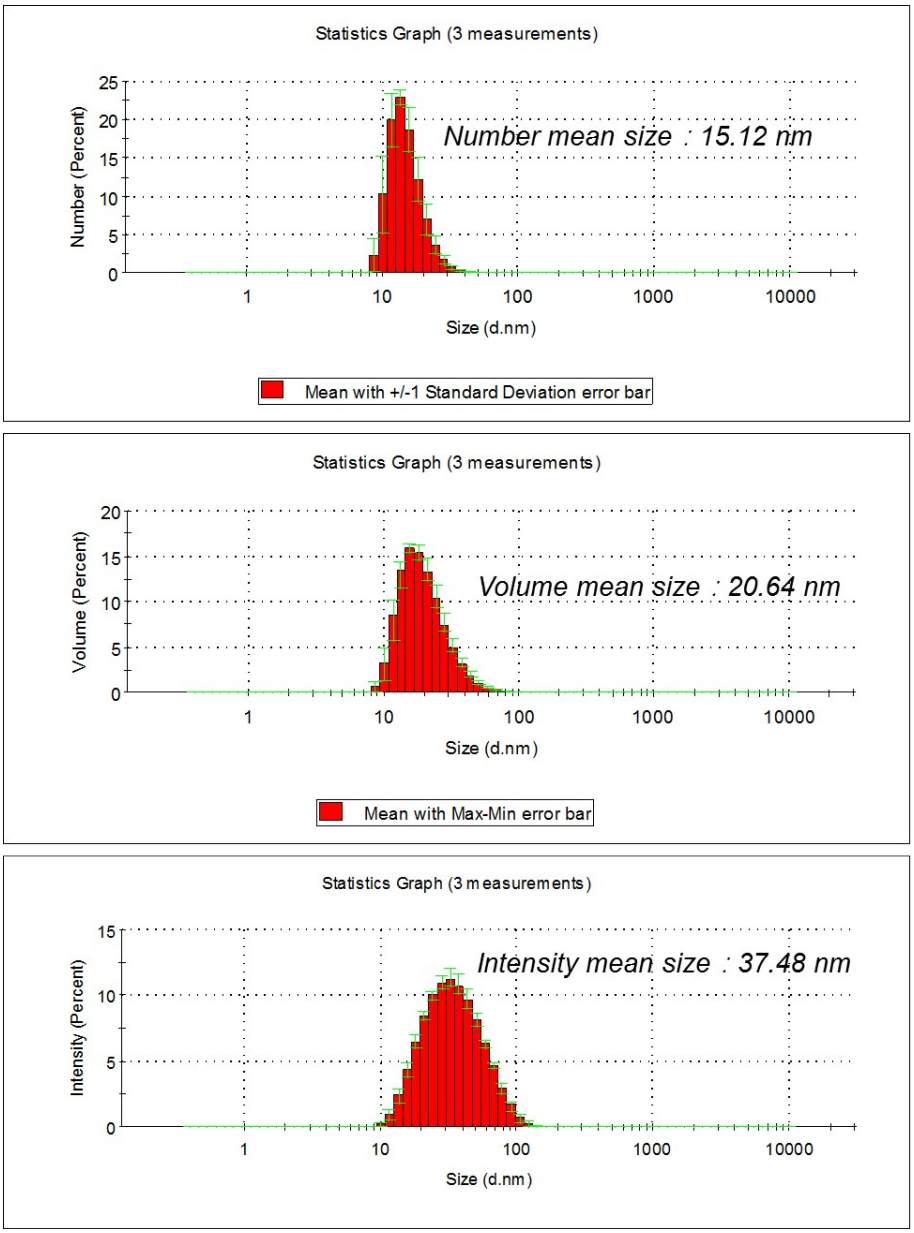


Figure S5. DLS determination of NIR-II AIEdots-1.

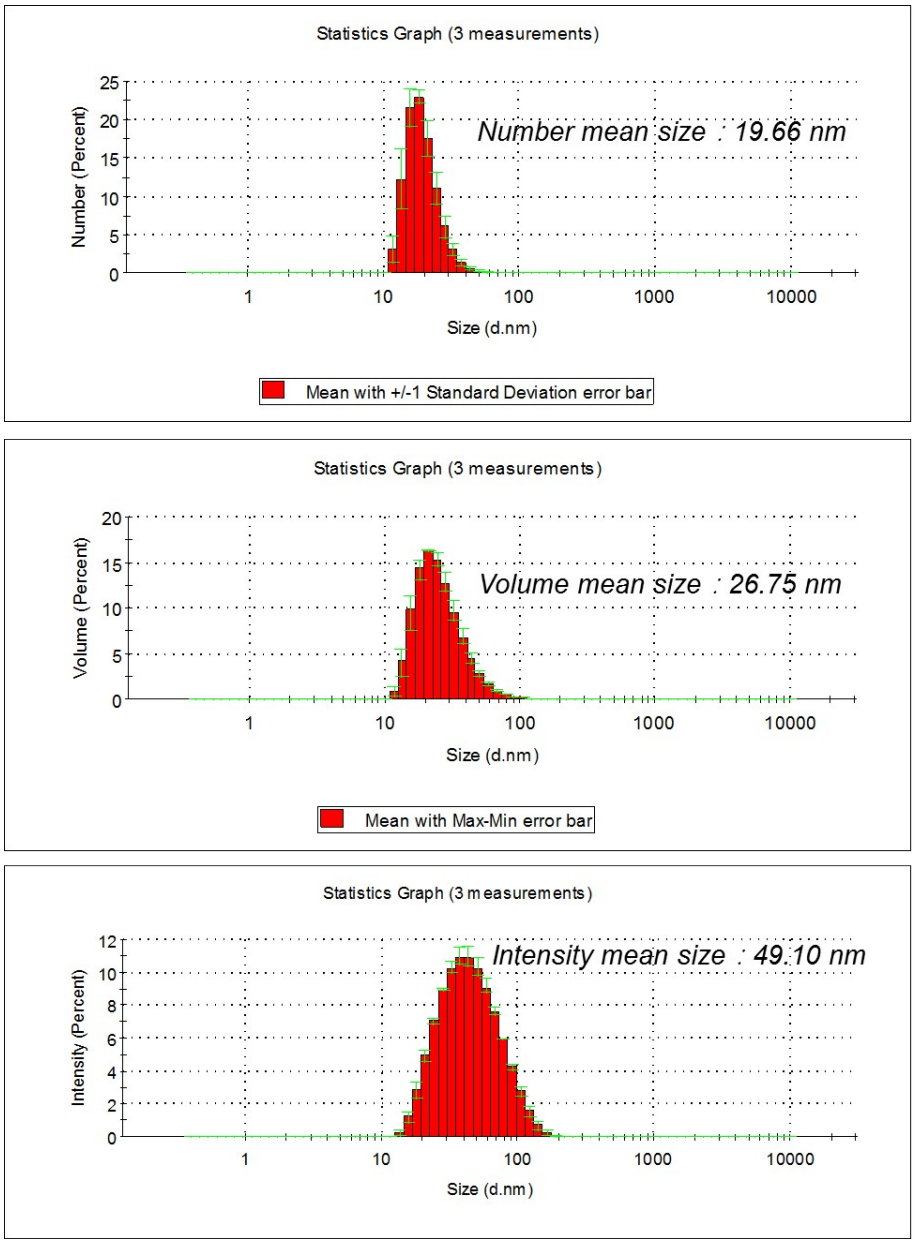


Figure S6. DLS determination of NIR-II AIEdots-2.

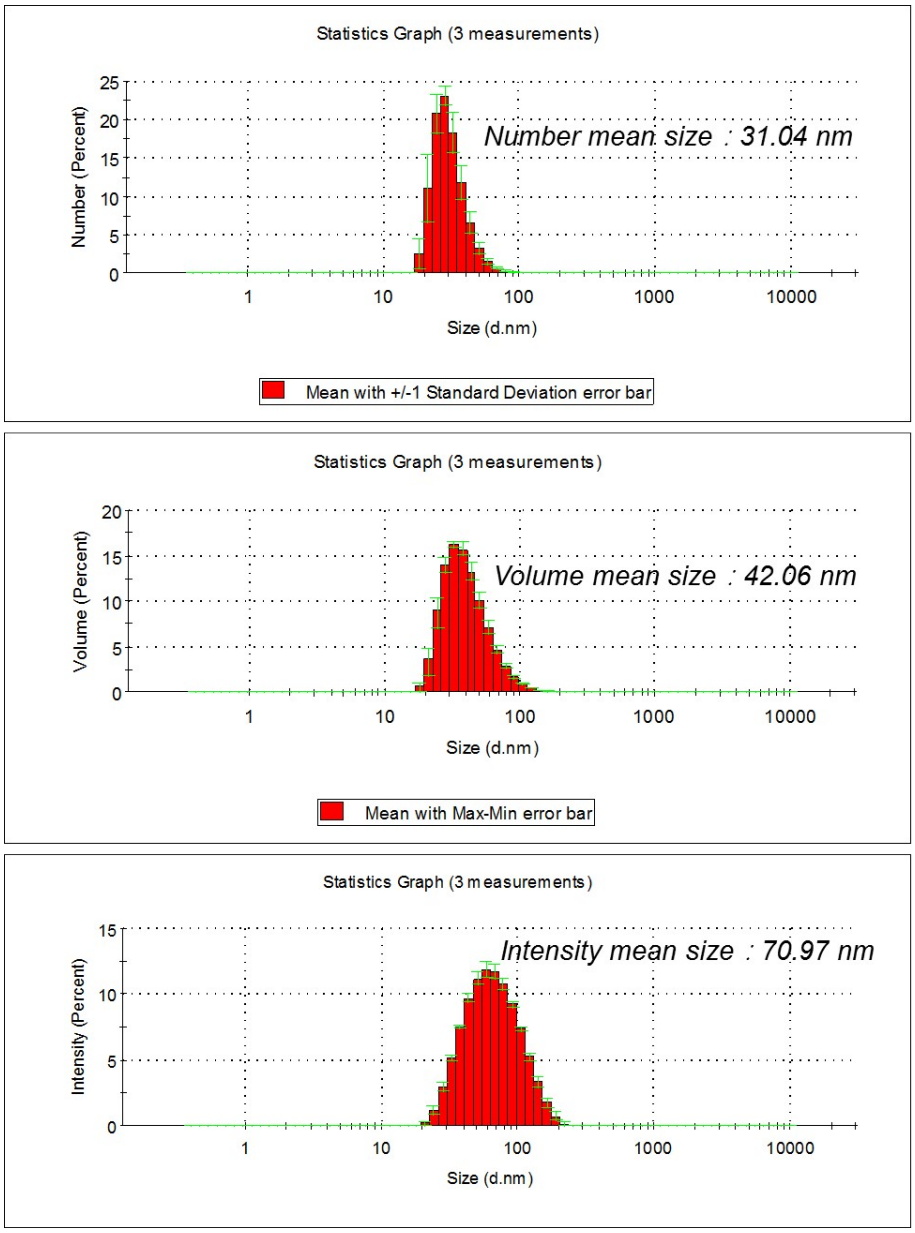


Figure S7. DLS determination of NIR-II AIEdots-3.

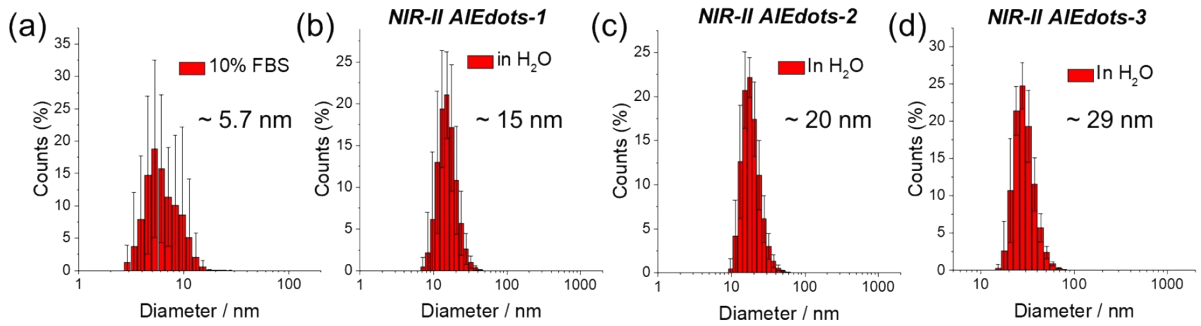


Figure S8. DLS determinations of a) FBS solution (V_{FBS}/V_{water}: 1/9) and different NIR-II AIEdots (b-NIR-II AIEdots-1, c-NIR-II AIEdots-2, d-NIR-II AIEdots-3) in water.

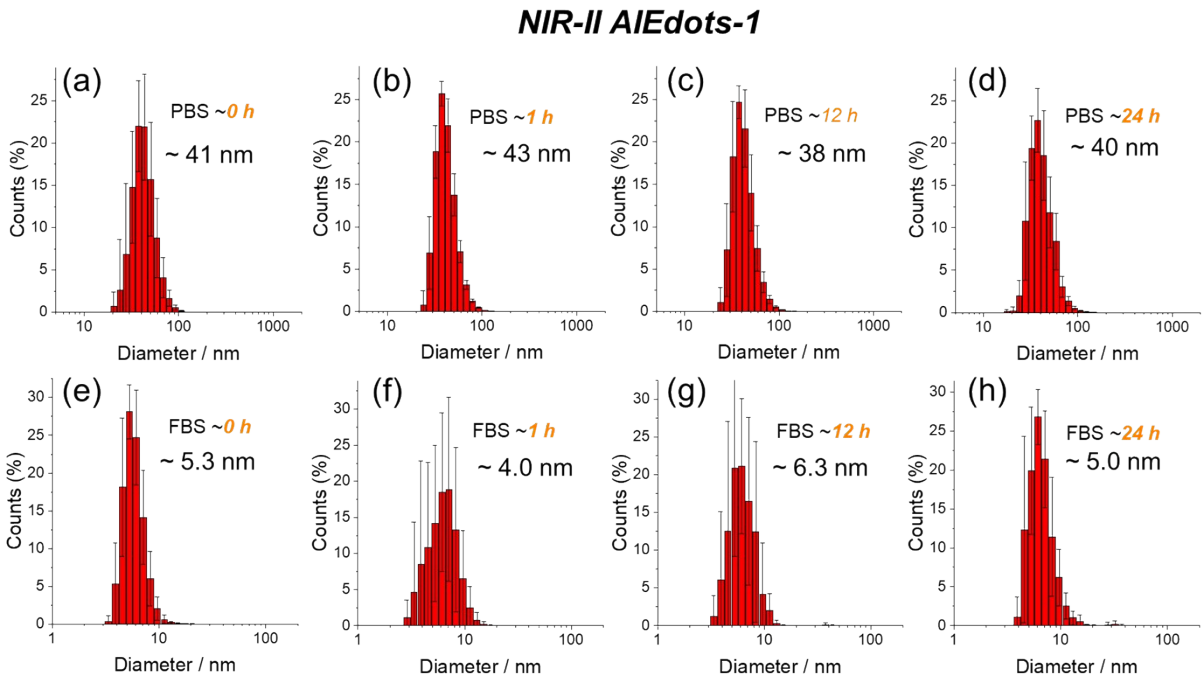


Figure S9. DLS determinations of NIR-II AIEdots-1 in pH 7.4 PBS solutions (a-d) and FBS solutions (e-h) at different period, respectively.

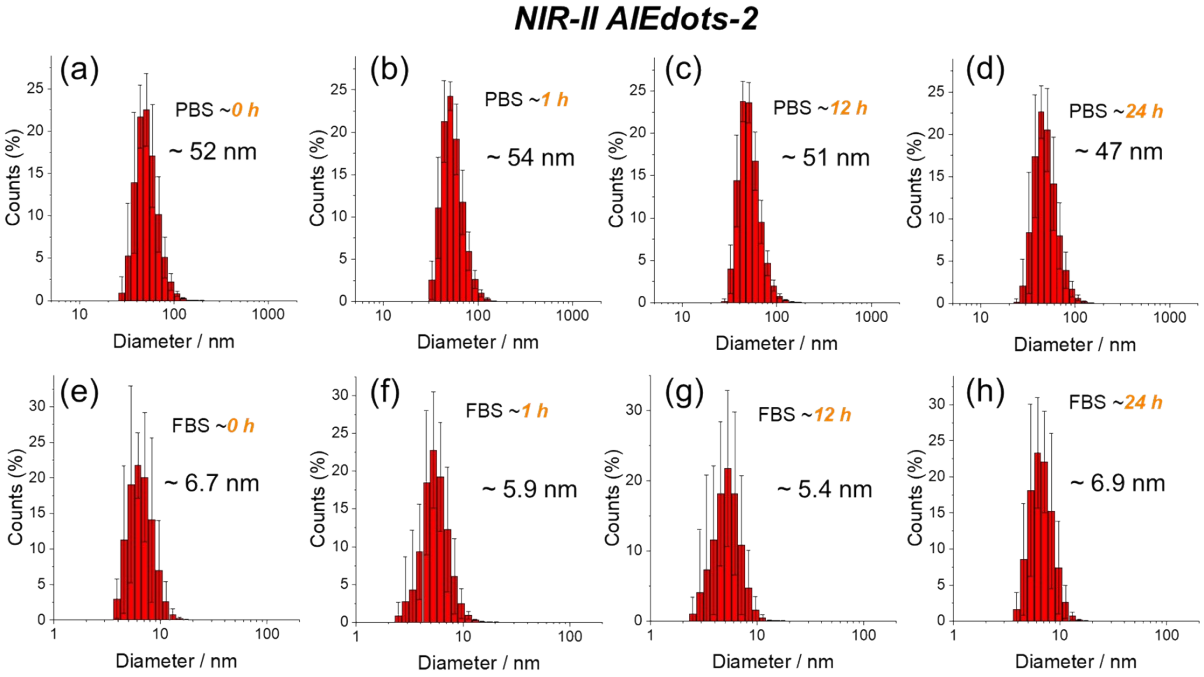


Figure S10. DLS determinations of NIR-II AIEdots-2 in pH 7.4 PBS solutions (a-d) and FBS solutions (e-h) at different period, respectively.

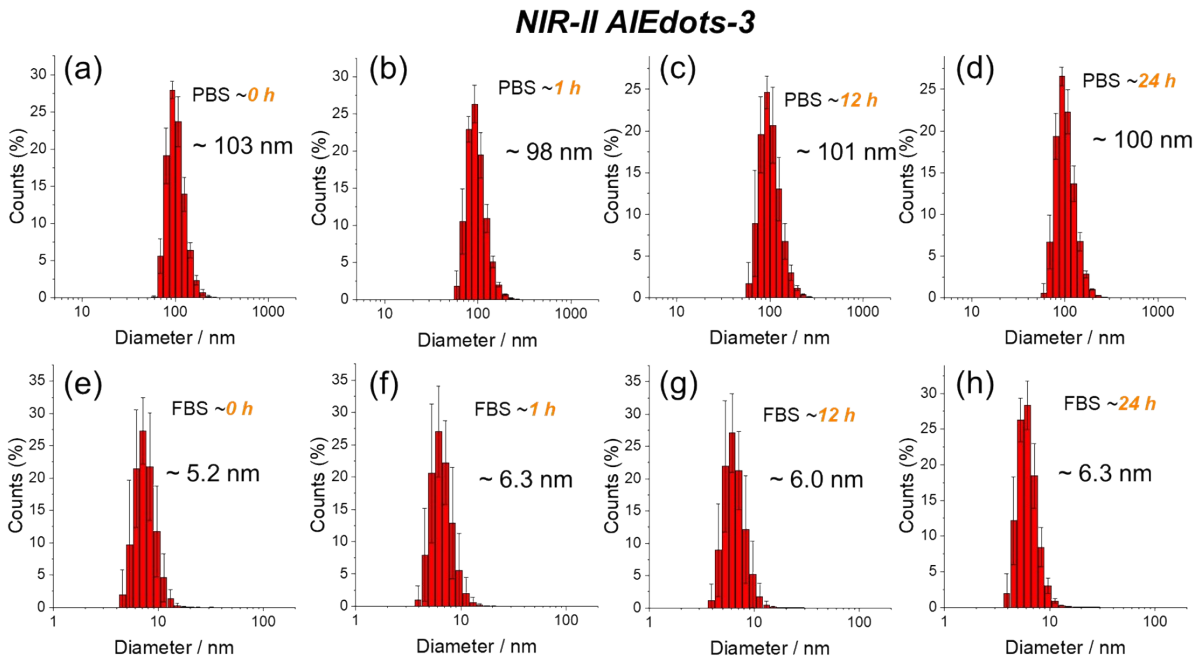


Figure S11. DLS determinations of NIR-II AIEdots-3 in pH 7.4 PBS solutions (a-d) and FBS solutions (e-h) at different period, respectively.

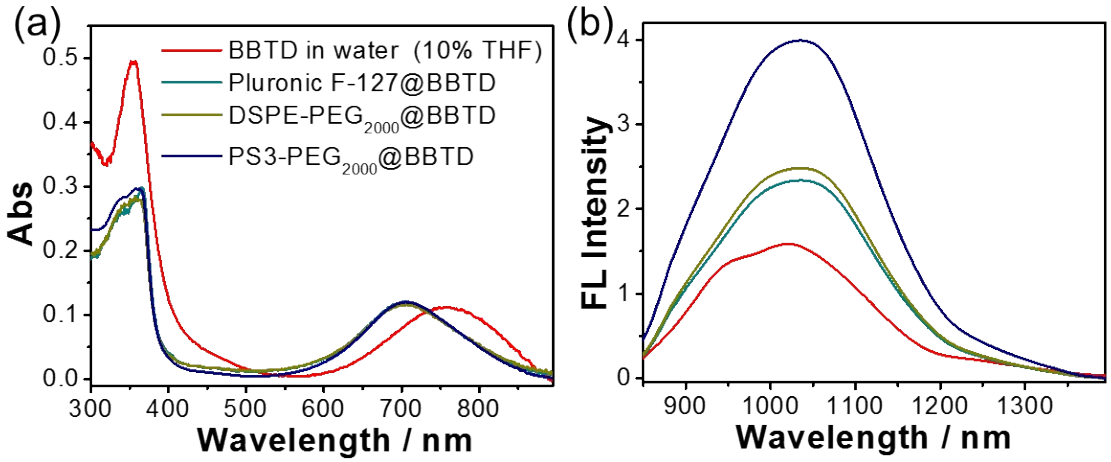


Figure S12. (a) Absorption and (b) emission spectra of the NIR-II AIEgen (TPA-BBTD) loaded by different amphiphilic polymers (PS3-PEG2000, DSPE-PEG2000 and pluronic F-127) disperse in water, and the NIR-II AIEgen directly dispersed in water (containing 10% content of THF).

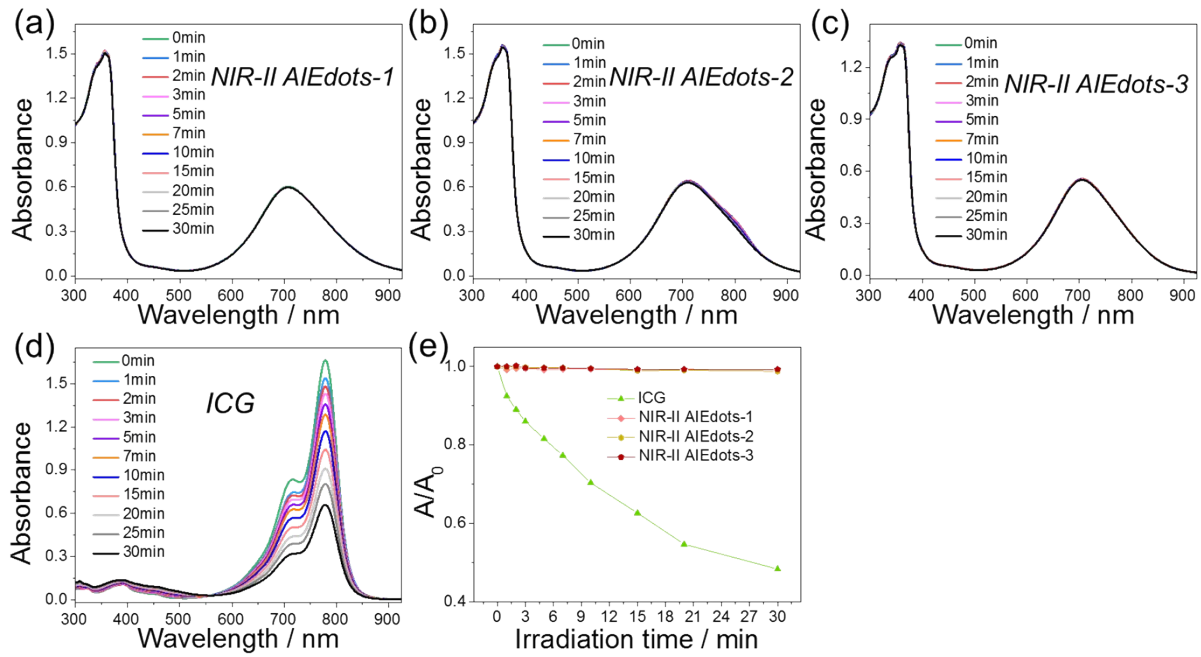


Figure S13. Photostability measurement of NIR-II AIEdots and ICG under the irradiation of 730 nm laser in water. Absorption spectra of a) NIR-II AIEdots-1, b) NIR-II AIEdots-2, c) NIR-II AIEdots-3 and d) ICG record at different time point after irradiation. (e) Variation of the absorbance of ICG at 780 nm and NIR-II AIEdots at 700 nm toward the variation of irradiation time.

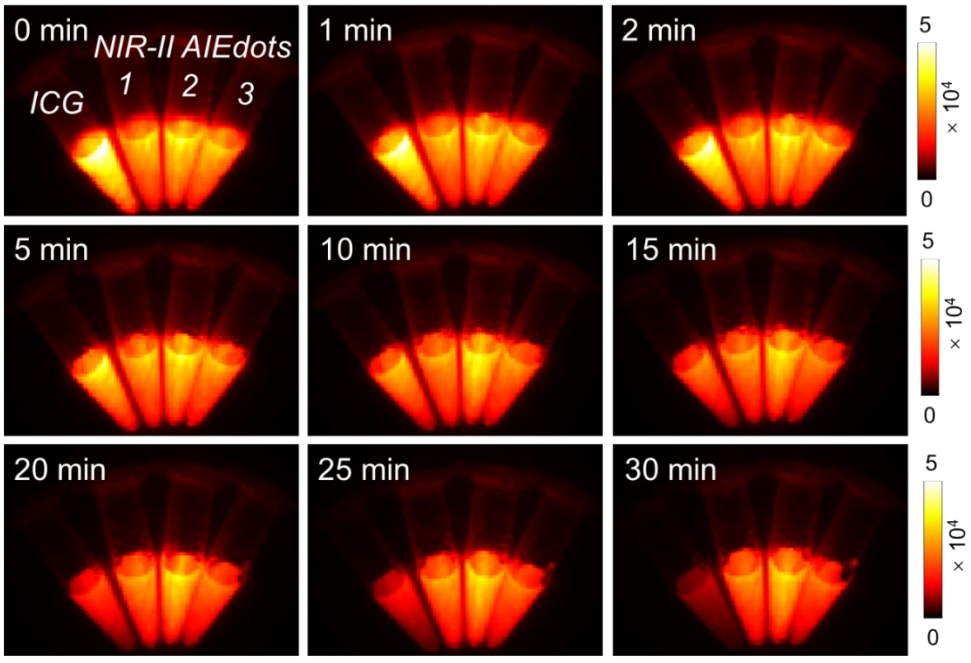


Figure S14. Fluorescence images of NIR-II AIEdots and ICG after irradiated by 730 nm laser for different period.

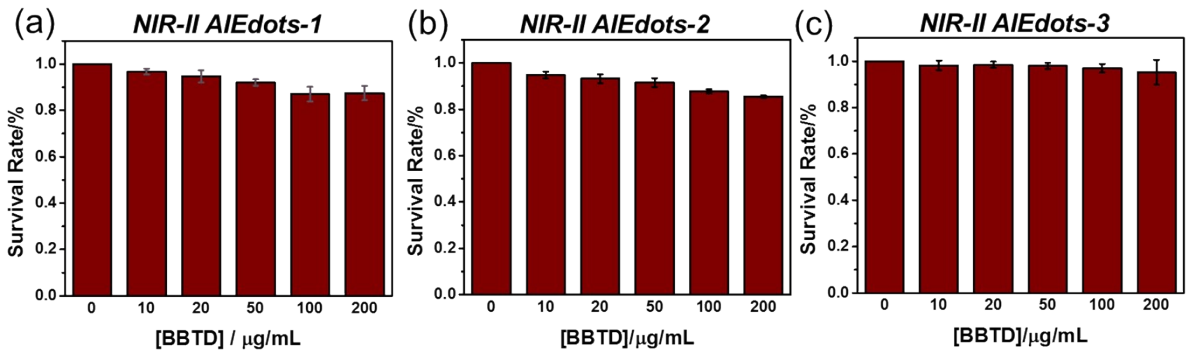


Figure S15. Cytotoxicity evaluation of NIR-II AIEdots-1~3 over Ags (human stomach adenocarcinoma) cell lines with 24 h incubation.

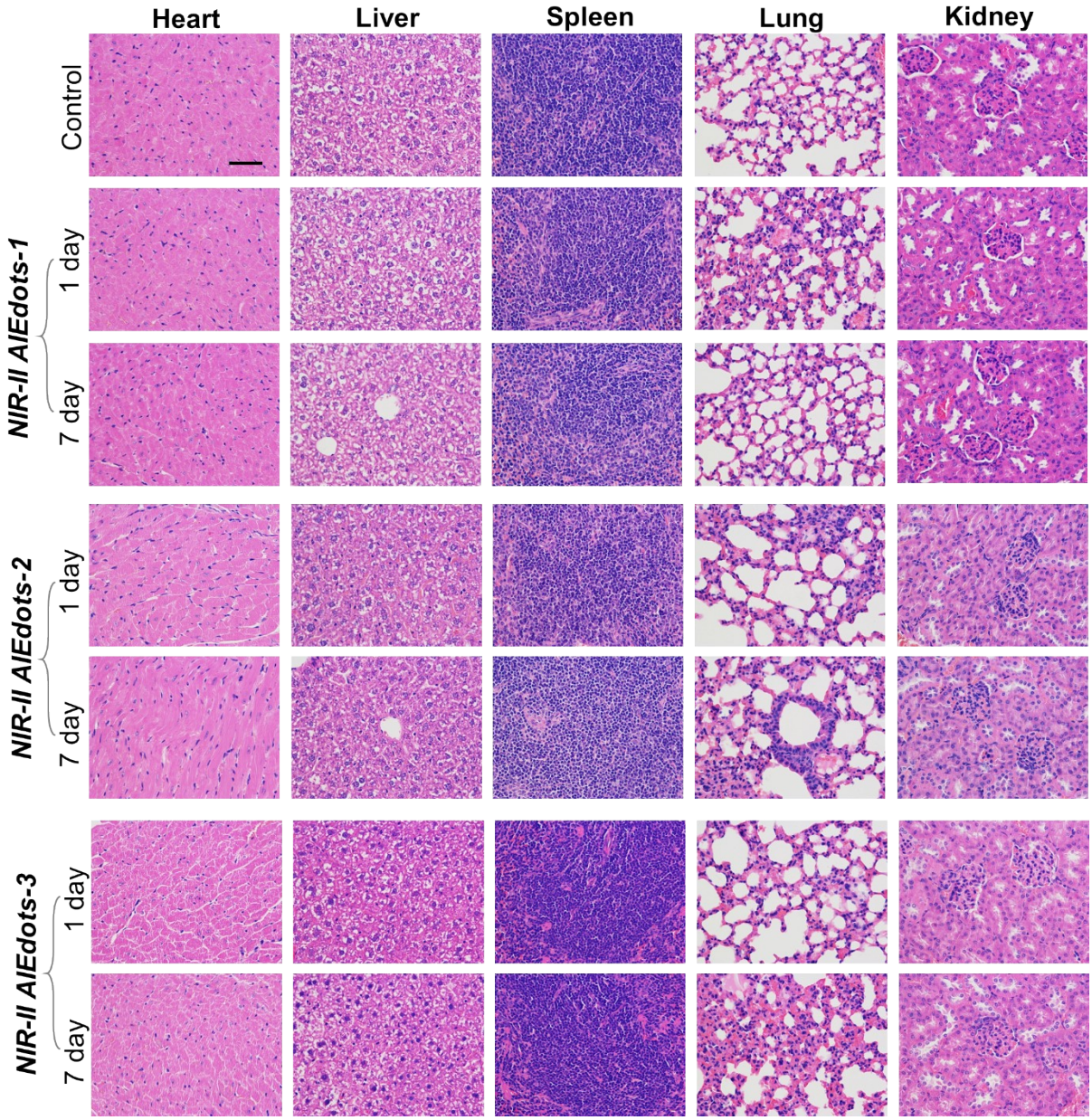


Figure S16. H&E-stained tissue sections of the heart, liver, spleen, lung and kidney harvested from mice after intravenous injection with PBS (control) or NIR-II AIEdots-1~3 (1 mg/mL, 200 μL). Bar: 100 μm

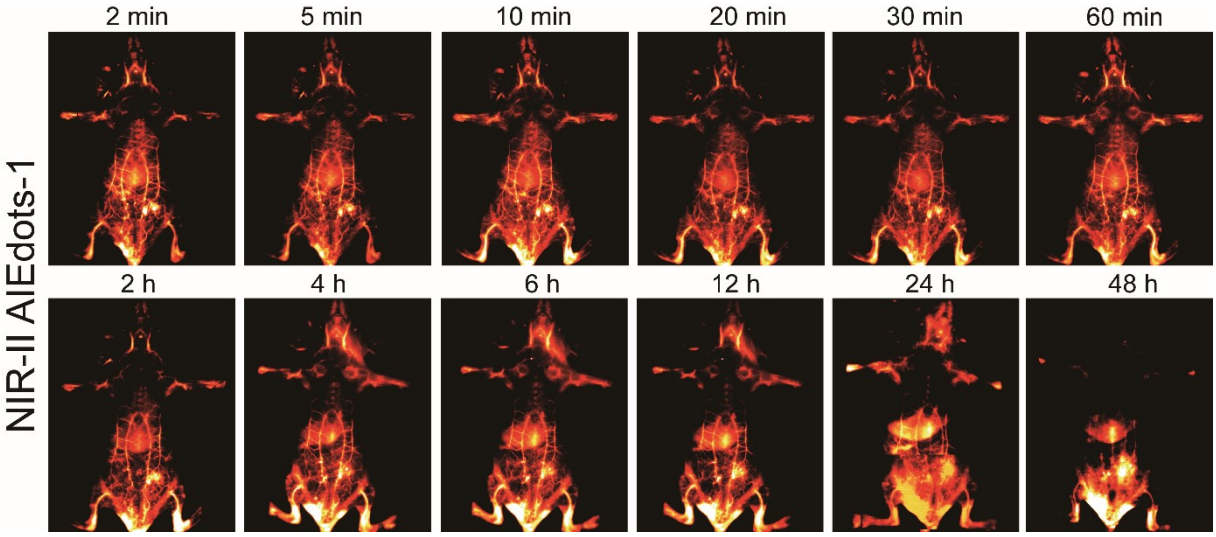


Figure S17. Time course of NIR-II fluorescence images of living mice I.V. injected with 200 μL (1 mg/mL) NIR-II AIEdots-1. Images were acquired at the time point of 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h and 48 h post-I.V. injection, respectively.

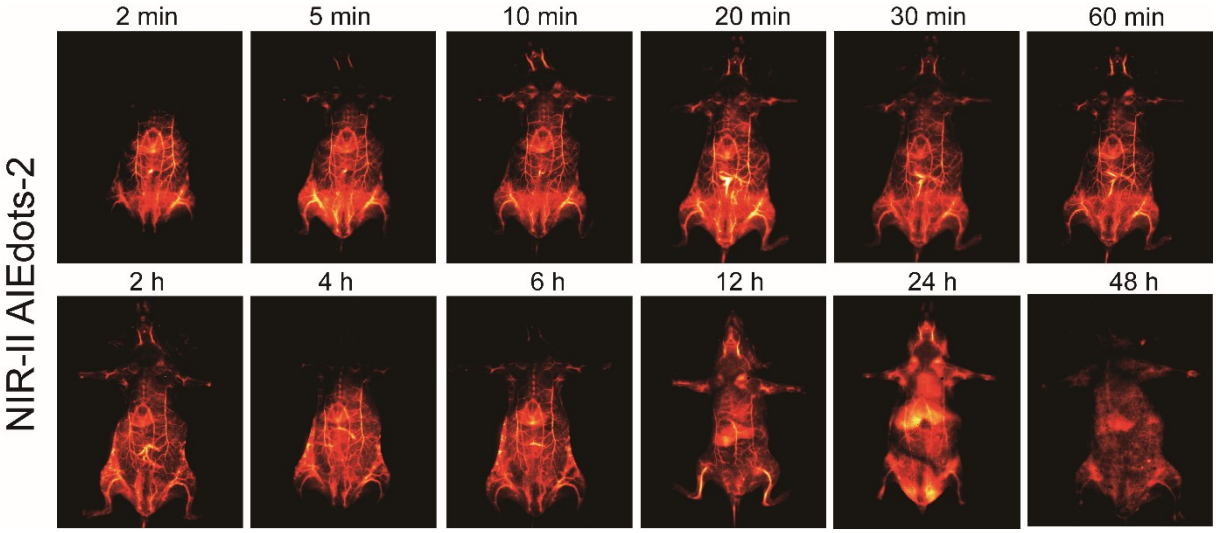


Figure S18. Time course of NIR-II fluorescence images of living mice I.V. injected with 200 μL (1 mg/mL) NIR-II AIEdots-2. Images were acquired at the time point of 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h and 48 h post-I.V. injection, respectively.

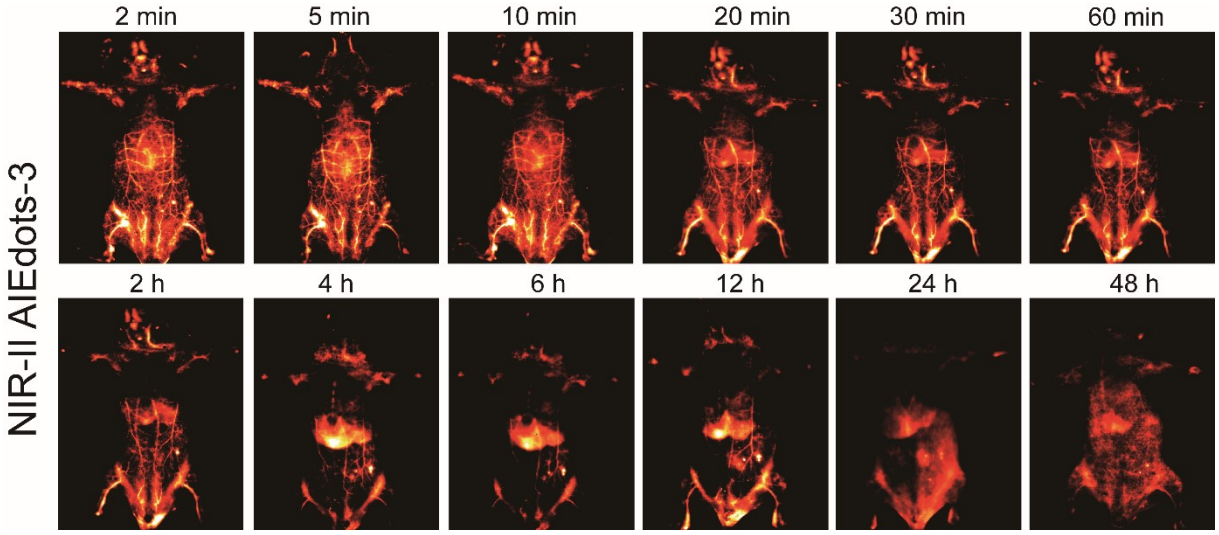


Figure S19. Time course of NIR-II fluorescence images of living mice I.V. injected with 200 μL (1 mg/mL) NIR-II AIEdots-3. Images were acquired at the time point of 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h and 48 h post-I.V. injection, respectively.

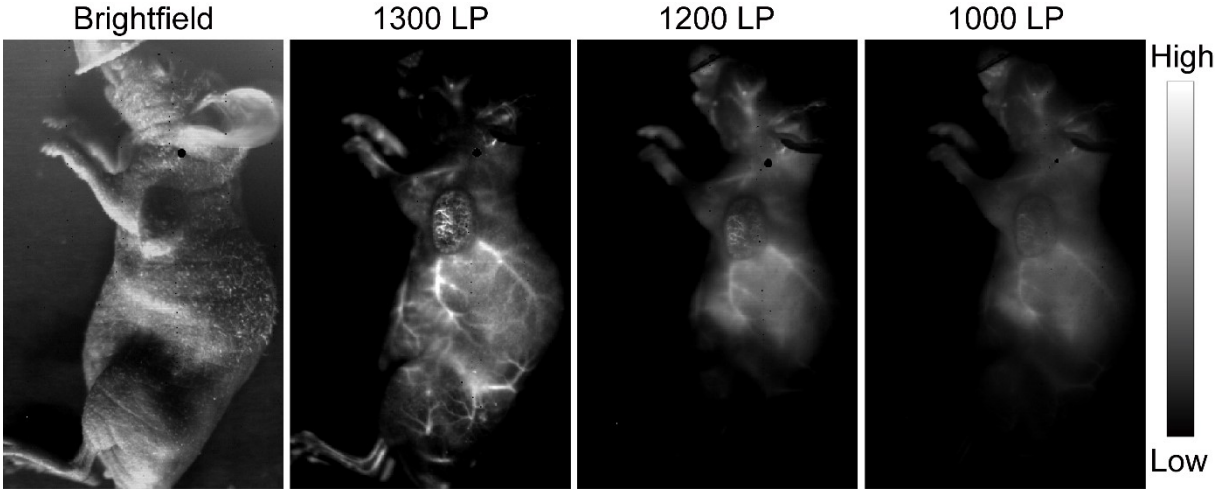


Figure S20. NIR-II fluorescence imaging of tumor vasculature performed on subcutaneous tumor-bearing mice under varying signal collection conditions (1000 LP, 1200 LP and 1300 LP) and the same excitation condition (excitation: 730 nm).

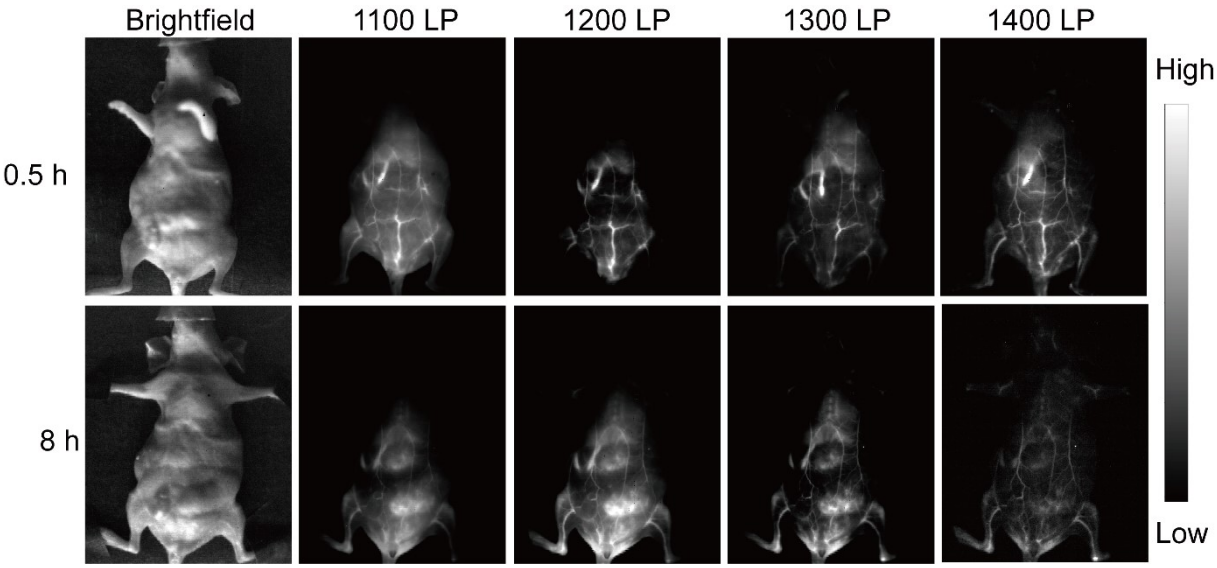


Figure S21. NIR-II fluorescence imaging performed on mice with abdominal metastases after I.V. injection of NIR-II AIEdots-2 at 0.5 h and 8 h, under varying signal collection conditions (1100 LP, 1200 LP, 1300 LP and 1400 LP), respectively.

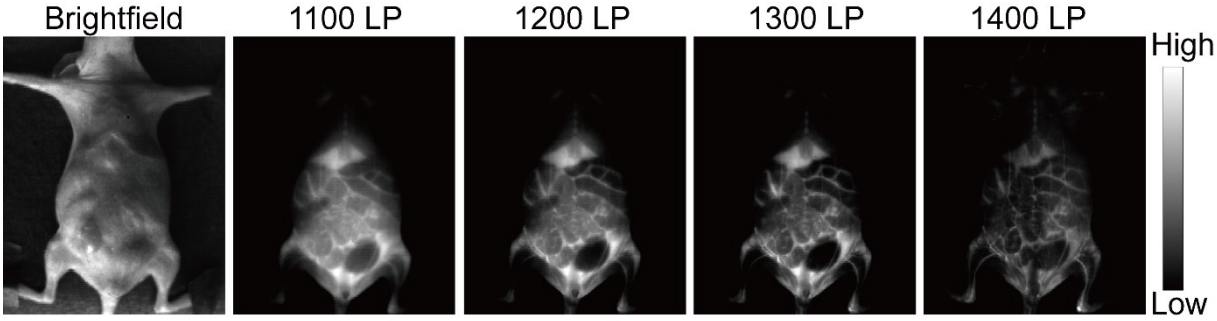


Figure S22. NIR-II fluorescence imaging performed on mice with abdominal metastases tumors 24 hours after I.V. injection of NIR-II AIEdots-2, under various signal collection conditions (1100 LP, 1200 LP, 1300 LP and 1400 LP), respectively.

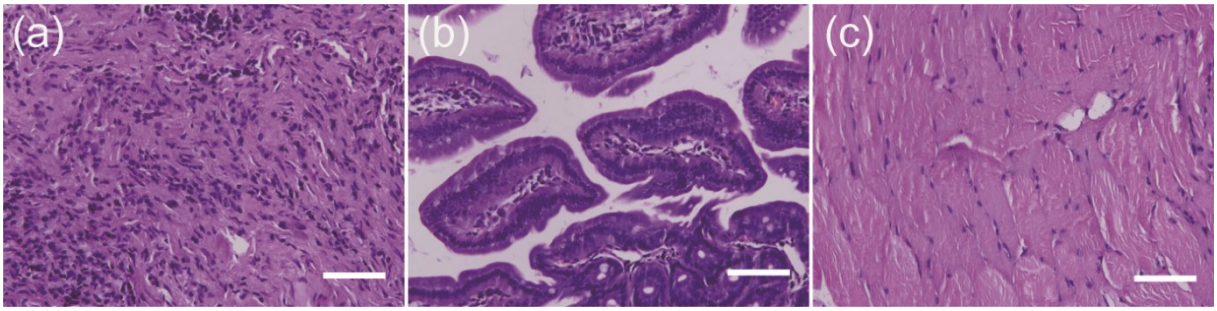
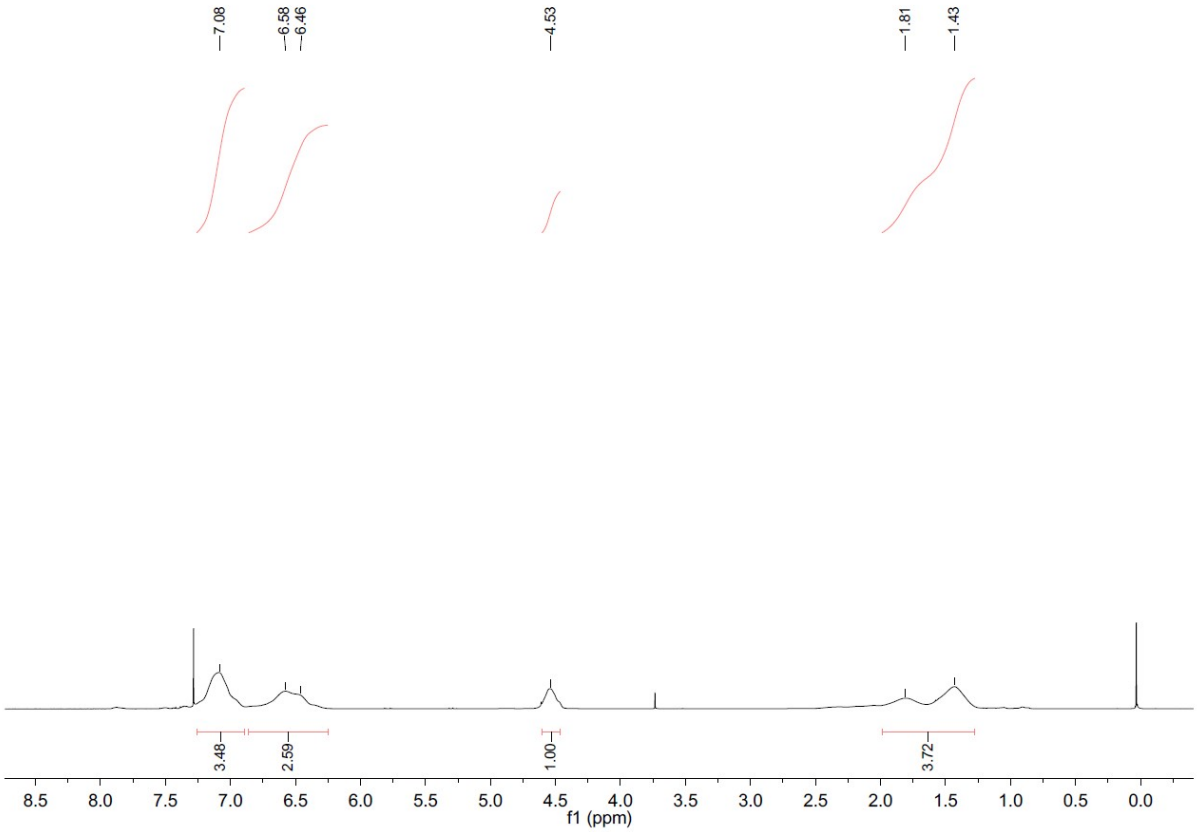
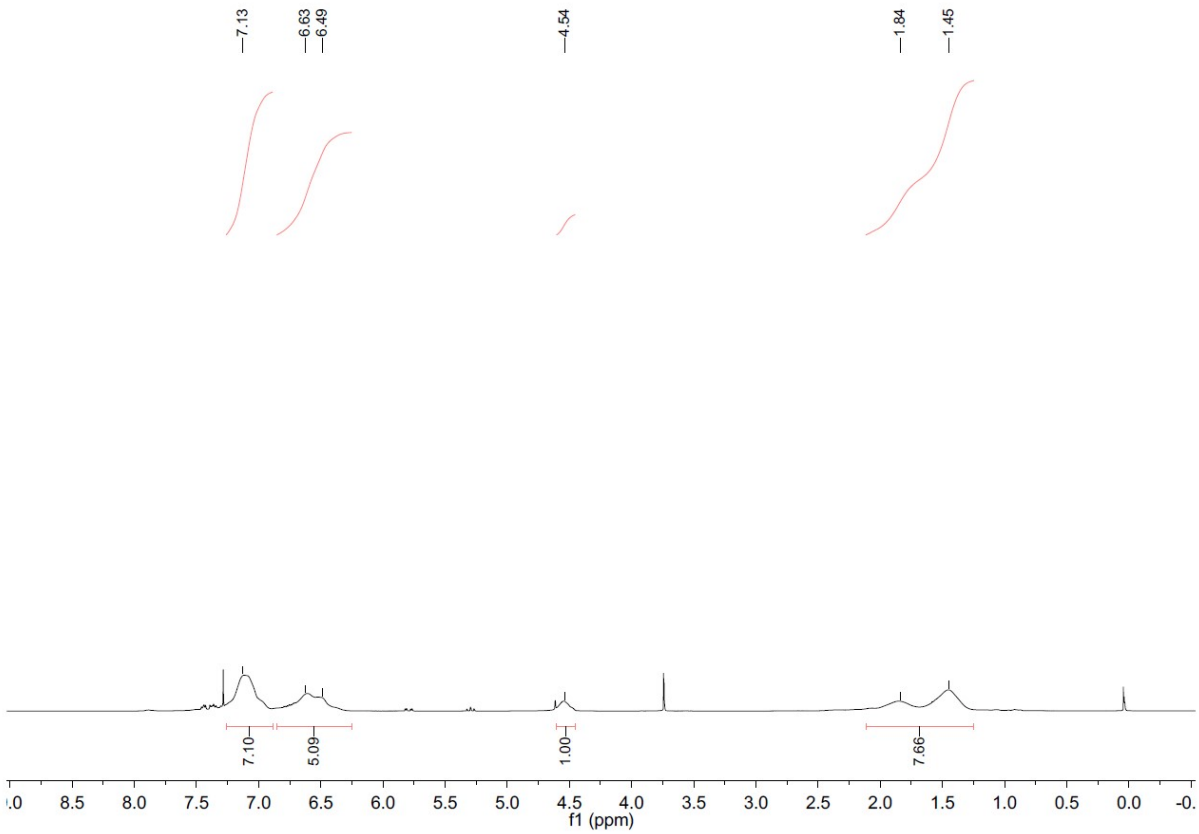


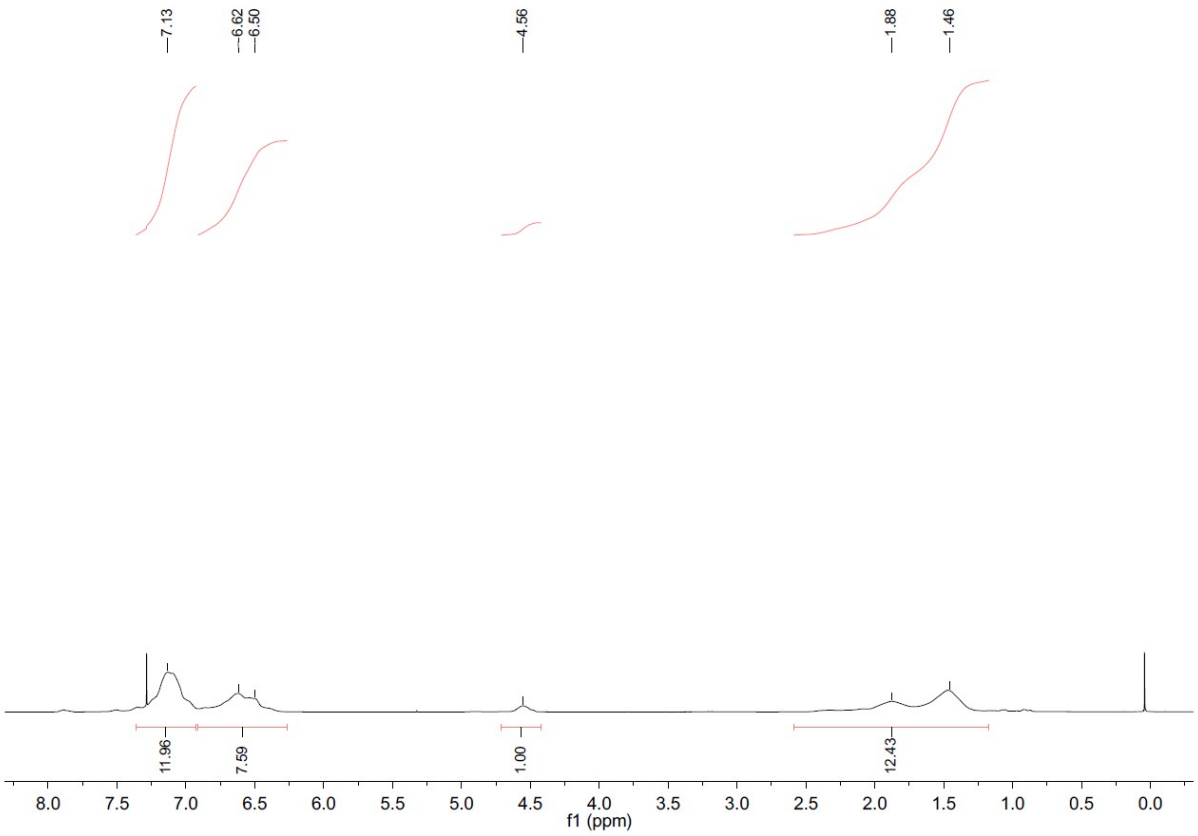
Figure S23. H&E staining analysis of the tissues (a-ROI 4, b-ROI 5 and c-ROI 6) labelled in Fig. 5c, respectively. Scale bar: 50 μm



^{1}H NMR of PS1 ($CDCl_3$)

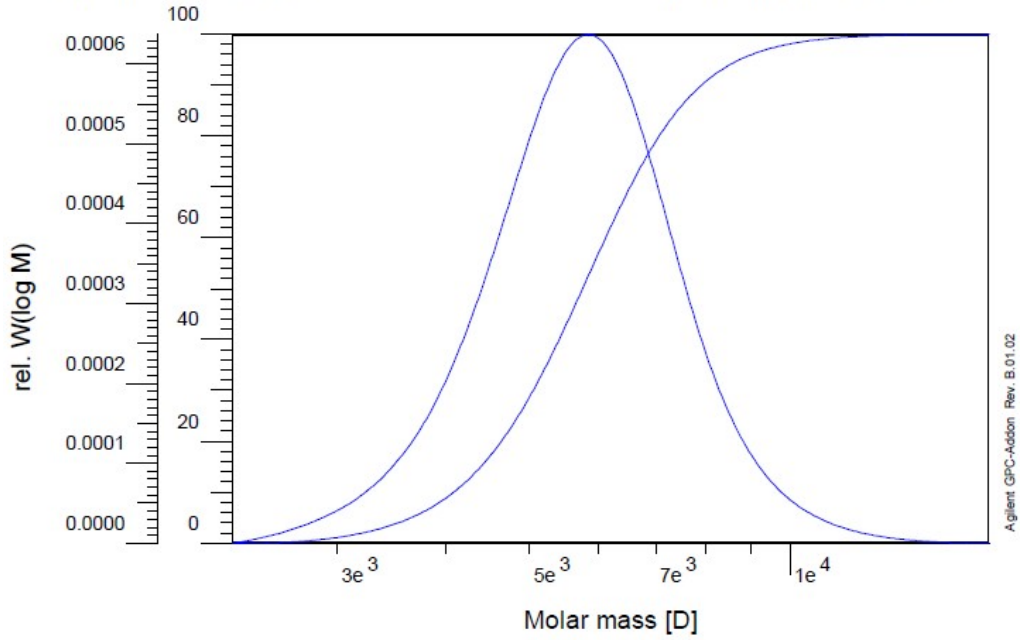


^{1}H NMR of PS2 ($CDCl_3$)



^{1}H NMR of PS3 ($CDCl_3$)

Injection Date :	26-Jul-18, 18:28:45		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.662 min	Baseline to :	26.050 min
Integration from:	22.662 min	Integration to :	26.050 min
MHK - A (Cal.):	0.000000E+0	MHK - K (Cal.):	1.000000E+0 ml/g
Eluent :		Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	Delay volume :	0.000 ml
Operator :	SYSTEM	Acquisition interval :	0.430 sec

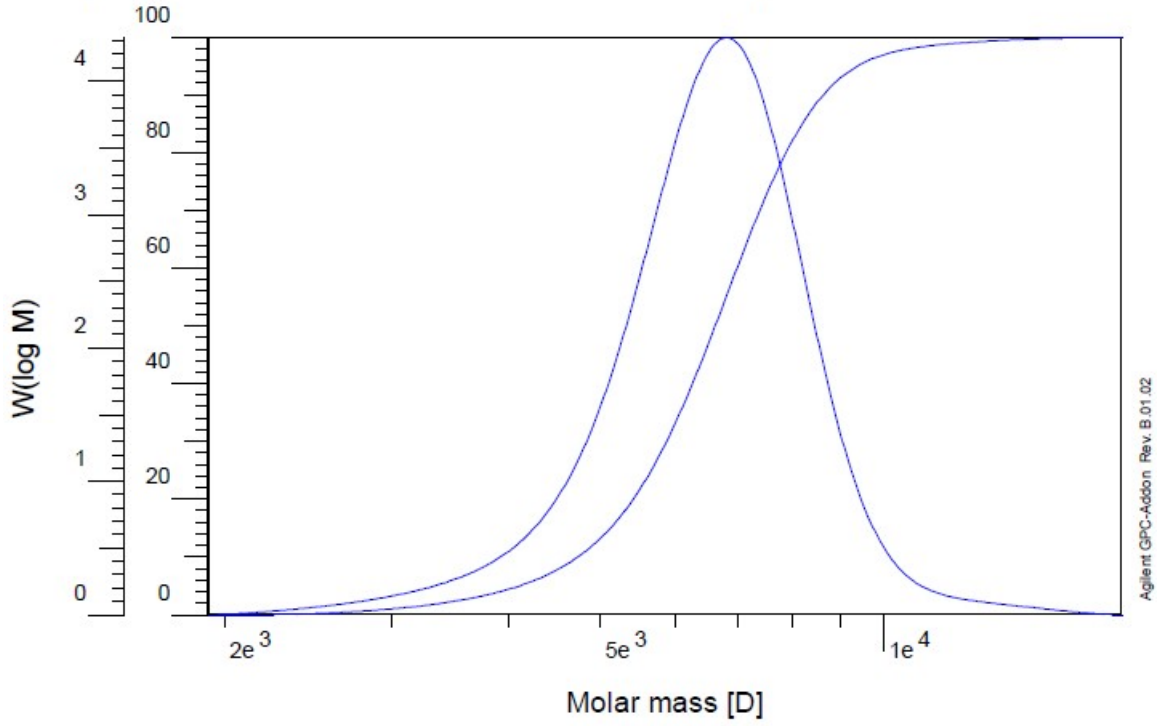


RID1A

Mn :	5.9176e3	g/mol
Mw :	6.3417e3	g/mol
Mz :	6.8048e3	g/mol
Mv :	0.000000	g/mol
D :	1.0717e0	
[n]:	0.000000	ml/g
Vp :	2.4358e1	ml
Mp :	6.1403e3	g/mol
A :	1.0432e4	ml*V
10%	4.0792e3	g/mol
30%	5.0461e3	g/mol
50%	5.7561e3	g/mol
70%	6.5325e3	g/mol
90%	7.8932e3	g/mol

GPC of PS1

Injection Date :	24-Apr-18, 15:20:08		
Calibration File :	E:\1METHOD\PS170310THF.CAL		
Calibration Date :	Wednesday 07/19/17 14:05:44		
Baseline from :	22.230 min	Baseline to :	25.941 min
Integration from:	22.230 min	Integration to :	25.941 min
MHK - A (Cal.):	0.000000E+0	MHK - K (Cal.):	1.000000E+0 ml/g
Eluent :		Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	Delay volume :	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	Delay volume :	0.000 ml
Operator :	SYSTEM	Acquisition interval :	0.430 sec

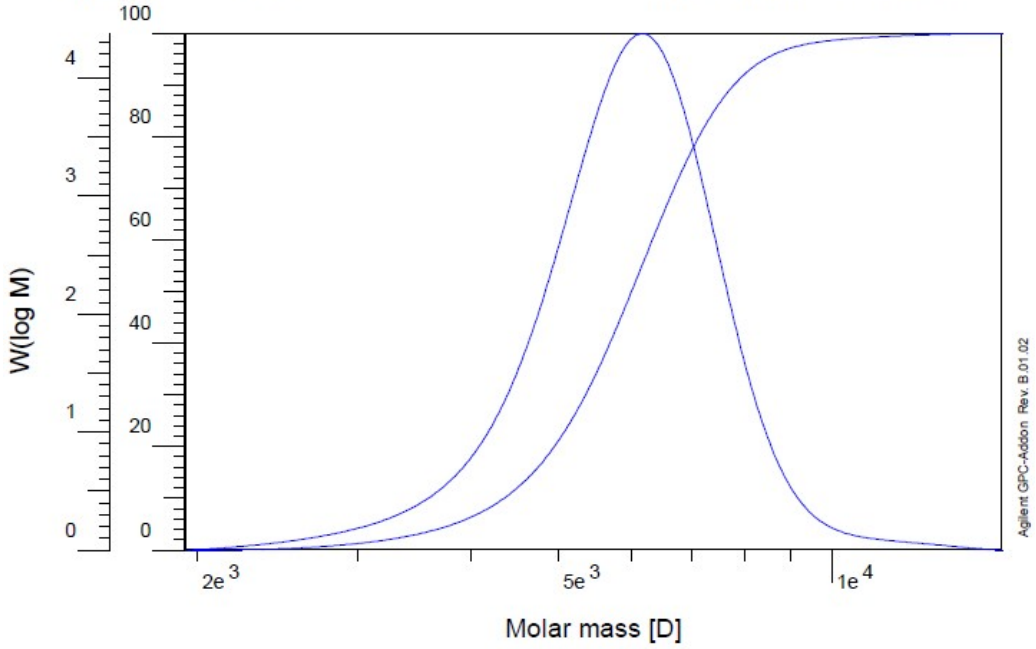


RID1A

Mn :	6.1540e3	g/mol
Mw :	6.5872e3	g/mol
Mz :	7.1054e3	g/mol
Mv :	0.000000	g/mol
D :	1.0693e0	
[n]:	0.000000	ml/g
Vp :	2.3839e1	ml
Mp :	6.8009e3	g/mol
A :	2.8523e4	ml*V
10%	4.7259e3	g/mol
30%	5.8762e3	g/mol
50%	6.6181e3	g/mol
70%	7.3791e3	g/mol
90%	8.6143e3	g/mol

GPC of PS2

Injection Date :	08-May-18, 14:38:20		
Calibration File :	E:\1METHOD\PS170310THF.CAL		
Calibration Date :	Wednesday 07/19/17 14:05:44		
Baseline from :	22.488 min	**Baseline to :**	25.926 min
Integration from:	22.488 min	**Integration to :**	25.926 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Detector 2 :	VWD1A, Wavelength=250 nm	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

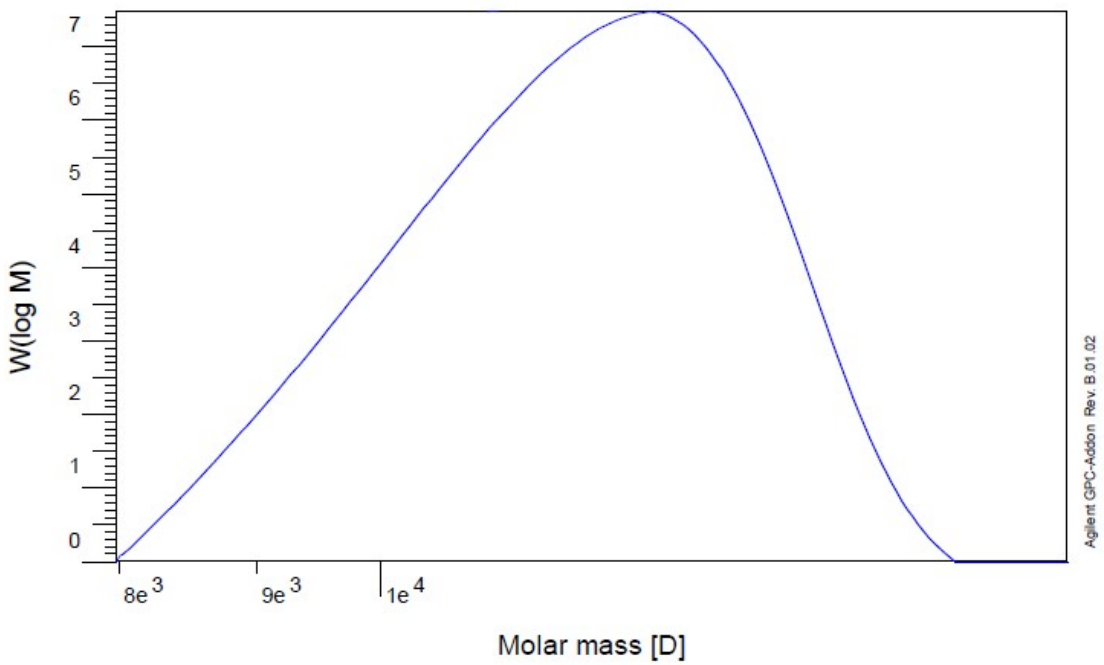


RID1A

Mn :	5.7119e3	g/mol
Mw :	6.0693e3	g/mol
Mz :	6.4176e3	g/mol
Mv :	0.000000	g/mol
D :	1.0626e0	
[n]:	0.000000	ml/g
Vp :	2.3998e1	ml
Mp :	6.1670e3	g/mol
A :	2.3609e4	ml*V
10%	4.3425e3	g/mol
30%	5.3442e3	g/mol
50%	6.0076e3	g/mol
70%	6.6900e3	g/mol
90%	7.7843e3	g/mol

GPC of PS3

Injection Date :	06-Aug-18, 21:41:44		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.567 min	**Baseline to :**	23.926 min
Integration from:	22.567 min	**Integration to :**	23.926 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

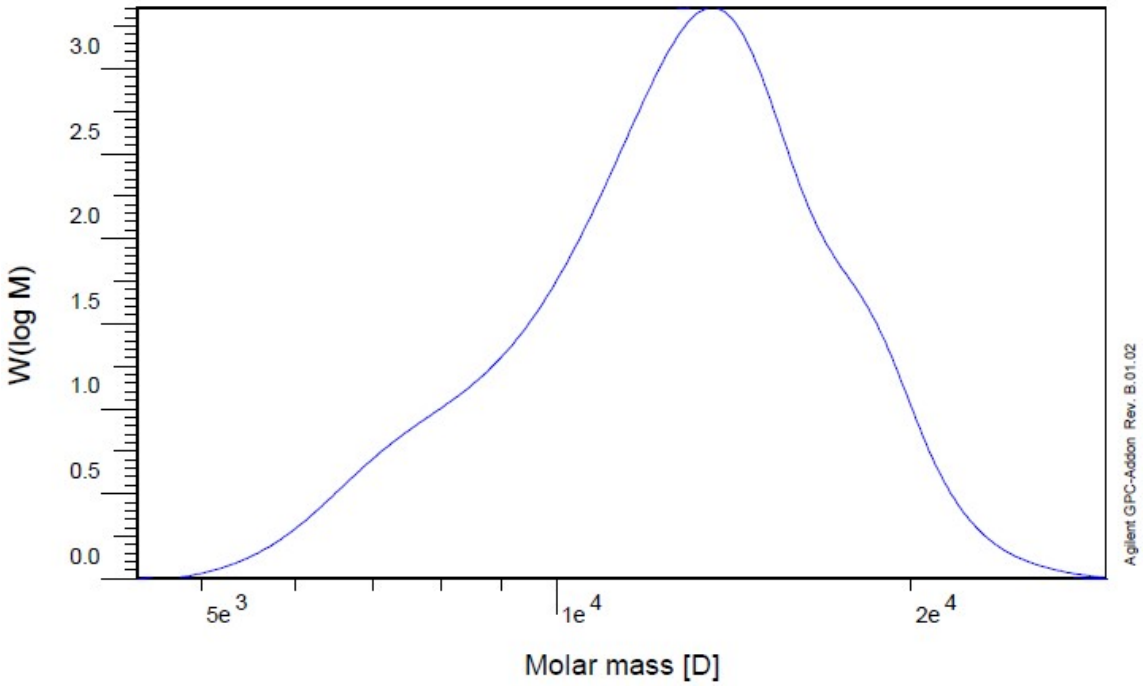


RID1A

Mn :	1.7333e4	g/mol
Mw :	1.9868e4	g/mol
Mz :	1.9901e4	g/mol
Mv :	0.000000	g/mol
D :	1.1426e0	
[n]:	0.000000	ml/g
Vp :	2.3393e1	ml
Mp :	1.8904e4	g/mol
A :	8.1702e3	ml*V
10%	1.5590e4	g/mol
30%	1.7466e4	g/mol
50%	1.8464e4	g/mol
70%	1.9454e4	g/mol
90%	2.0364e4	g/mol

GPC of PS1- PEG1000

Injection Date :	07-Aug-18, 10:50:11		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	21.720 min	**Baseline to :**	24.910 min
Integration from:	21.720 min	**Integration to :**	24.910 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

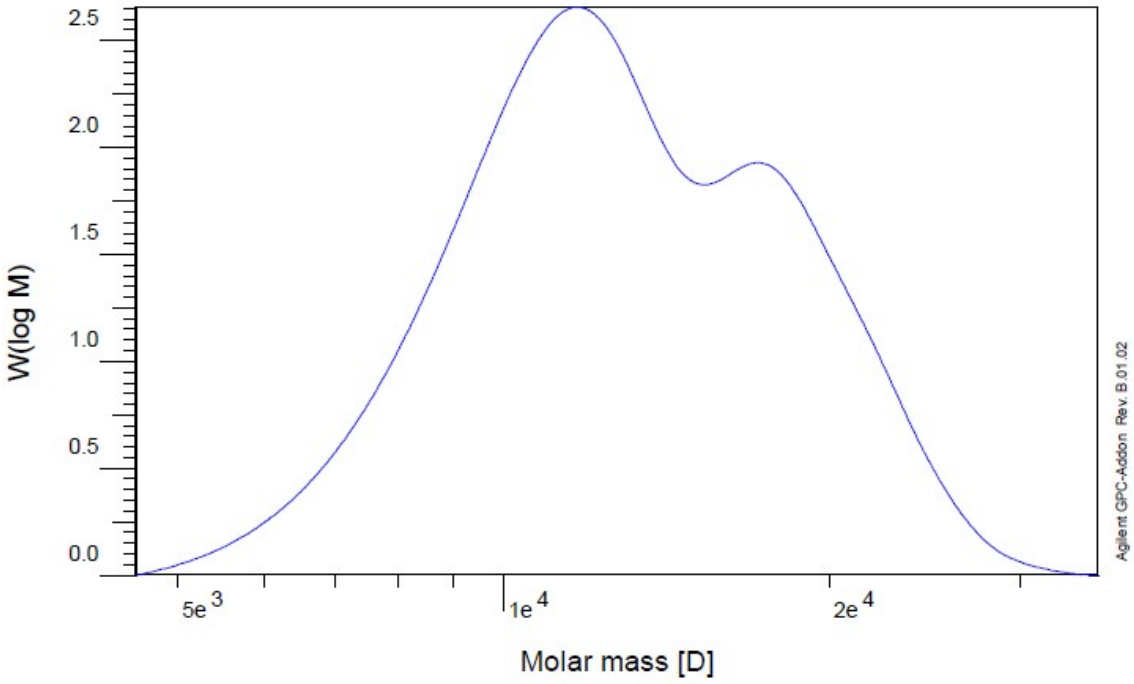


RID1A

Mn :	1.5151e4	g/mol
Mw :	1.6542e4	g/mol
Mz :	1.7900e4	g/mol
Mv :	0.000000	g/mol
D :	1.0970e0	
[n]:	0.000000	ml/g
Vp :	2.3141e1	ml
Mp :	1.2795e4	g/mol
A :	3.0403e4	ml*V
10%	7.6402e3	g/mol
30%	1.0319e4	g/mol
50%	1.2166e4	g/mol
70%	1.4002e4	g/mol
90%	1.7207e4	g/mol

GPC of PS2-PEG1000

Injection Date :	07-Aug-18, 11:32:46		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	21.385 min	**Baseline to :**	24.851 min
Integration from:	21.385 min	**Integration to :**	24.851 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Detector 2 :	VWD1A, Wavelength=400 nm	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

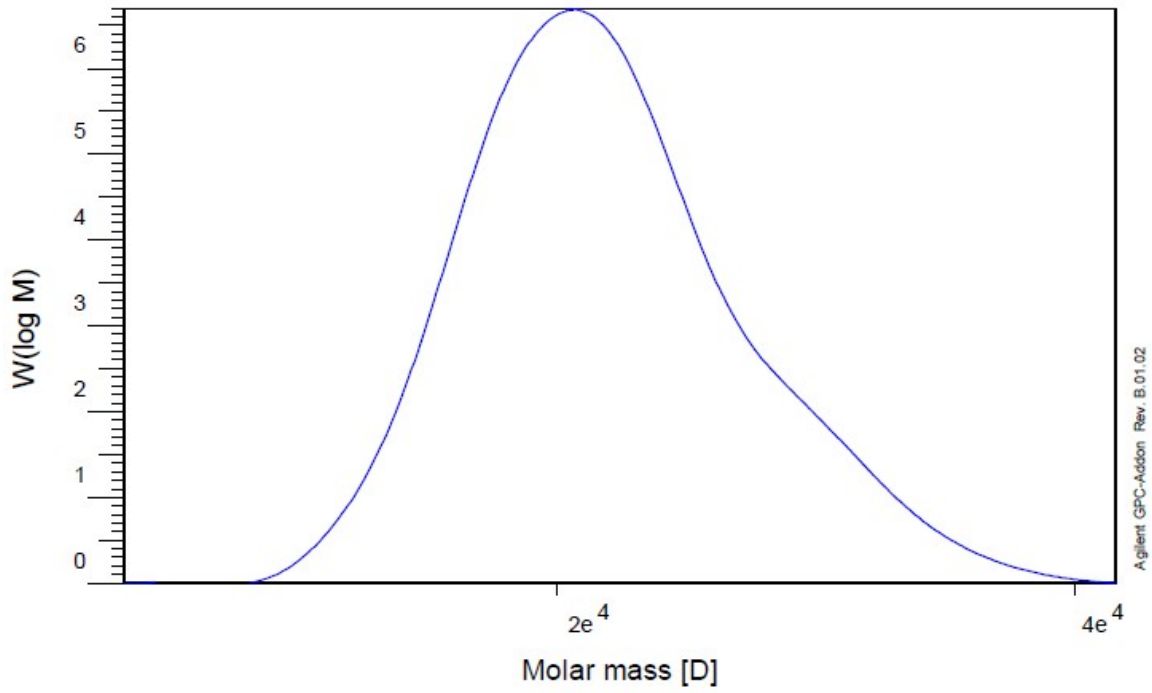


RID1A

Mn :	1.2145e4	g/mol
Mw :	1.3688e4	g/mol
Mz :	1.5356e4	g/mol
Mv :	0.000000	g/mol
D :	1.1271e0	
[n]:	0.000000	ml/g
Vp :	2.3292e1	ml
Mp :	1.1690e4	g/mol
A :	4.1637e4	ml*V
10%	8.2541e3	g/mol
30%	1.0613e4	g/mol
50%	1.2673e4	g/mol
70%	1.5853e4	g/mol
90%	2.0541e4	g/mol

GPC of PS3-PEG1000

Injection Date :	26-Jul-18, 16:21:06		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.080 min	Baseline to :	24.693 min
Integration from:	22.080 min	Integration to :	24.693 min
MHK - A (Cal.):	0.000000E+0	MHK - K (Cal.):	1.000000E+0 ml/g
Eluent :		Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	Delay volume :	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	Delay volume :	0.000 ml
Operator :	SYSTEM	Acquisition interval :	0.430 sec

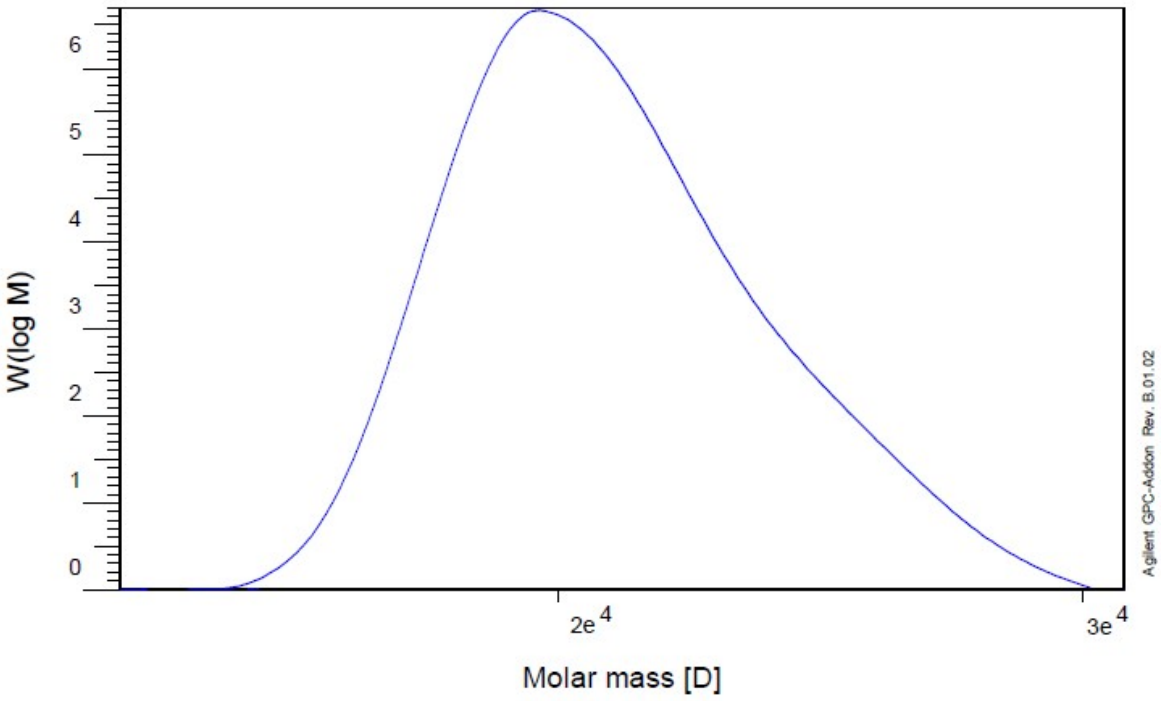


RID1A

Mn :	2.0092e4	g/mol
Mw :	2.4081e4	g/mol
Mz :	2.5783e4	g/mol
Mv :	0.000000	g/mol
D :	1.1985e0	
[n]:	0.000000	ml/g
Vp :	2.3731e1	ml
Mp :	2.4721e4	g/mol
A :	5.0290e3	ml*V
10%	1.6744e4	g/mol
30%	1.9282e4	g/mol
50%	2.1659e4	g/mol
70%	2.8169e4	g/mol
90%	3.4810e4	g/mol

GPC of PS1-PEG2000

Injection Date :	06-Aug-18, 20:07:40		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.864 min	**Baseline to :**	24.519 min
Integration from:	22.864 min	**Integration to :**	24.519 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

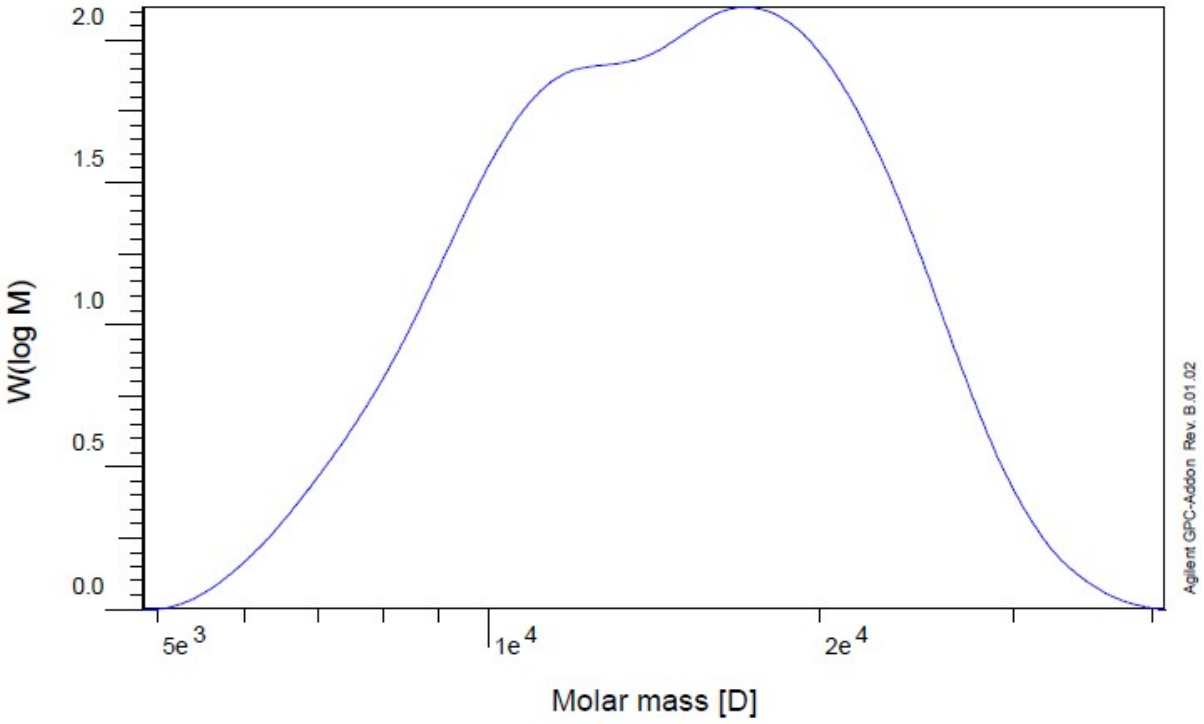


RID1A

Mn :	1.7089e4	g/mol
Mw :	1.9973e4	g/mol
Mz :	2.2794e4	g/mol
Mv :	0.000000	g/mol
D :	1.1688e0	
[n]:	0.000000	ml/g
Vp :	2.3674e1	ml
Mp :	2.0904e4	g/mol
A :	4.0411e3	ml*V
10%	1.4617e4	g/mol
30%	1.6035e4	g/mol
50%	1.9533e4	g/mol
70%	2.2196e4	g/mol
90%	2.7693e4	g/mol

GPC of PS2-PEG2000

Injection Date :	28-Nov-18, 09:49:04		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Monday 09/03/18 14:31:50		
Baseline from :	21.130 min	Baseline to :	24.752 min
Integration from:	21.130 min	Integration to :	24.752 min
MHK - A (Cal.):	0.000000E+0	MHK - K (Cal.):	1.000000E+0 ml/g
Eluent :		Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	Delay volume :	0.000 ml
Detector 2 :	VWD1A, Wavelength=250 nm	Delay volume :	0.000 ml
Operator :	SYSTEM	Acquisition interval :	0.430 sec

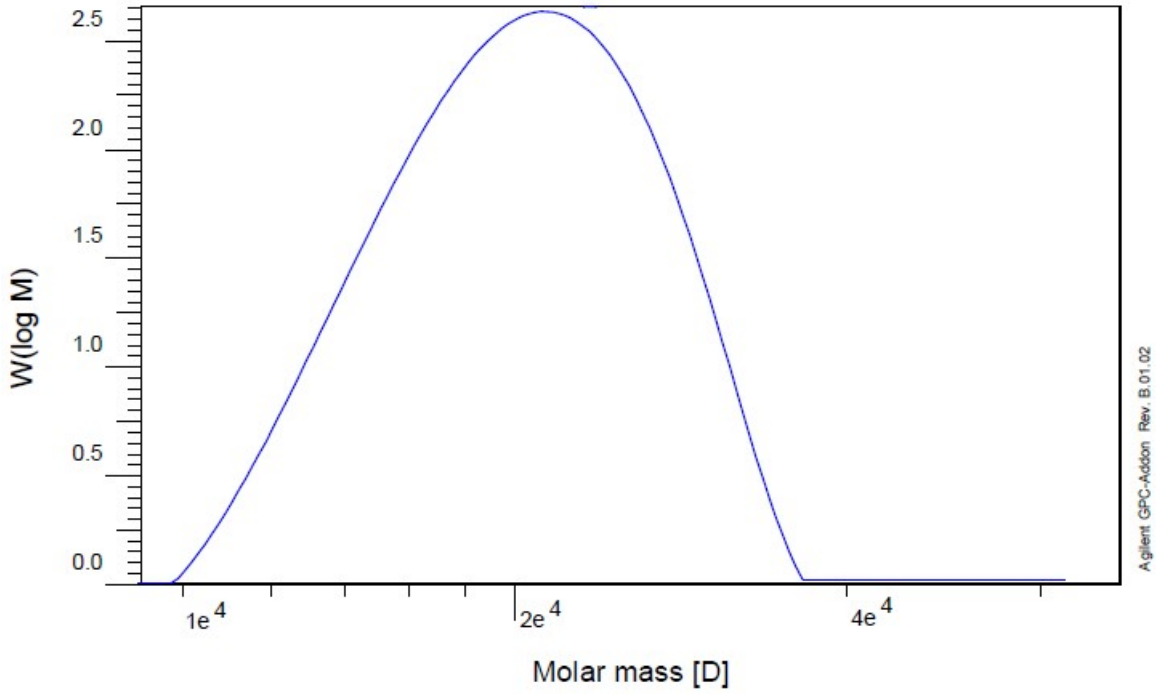


RID1A

$\overline{Mn}$:	1.3636e4	g/mol
$\overline{Mw}$:	1.5765e4	g/mol
$\overline{Mz}$:	1.8000e4	g/mol
$\overline{Mv}$:	0.000000	g/mol
D :	1.1561e0	
[n]:	0.000000	ml/g
Vp :	2.2666e1	ml
Mp :	1.6947e4	g/mol
A :	3.6295e3	ml*V
10%	8.7595e3	g/mol
30%	1.1750e4	g/mol
50%	1.4910e4	g/mol
70%	1.8588e4	g/mol
90%	2.4076e4	g/mol

GPC of PS3-PEG2000

Injection Date :	06-Aug-18, 17:01:20		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.094 min	**Baseline to :**	23.158 min
Integration from:	22.094 min	**Integration to :**	23.158 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

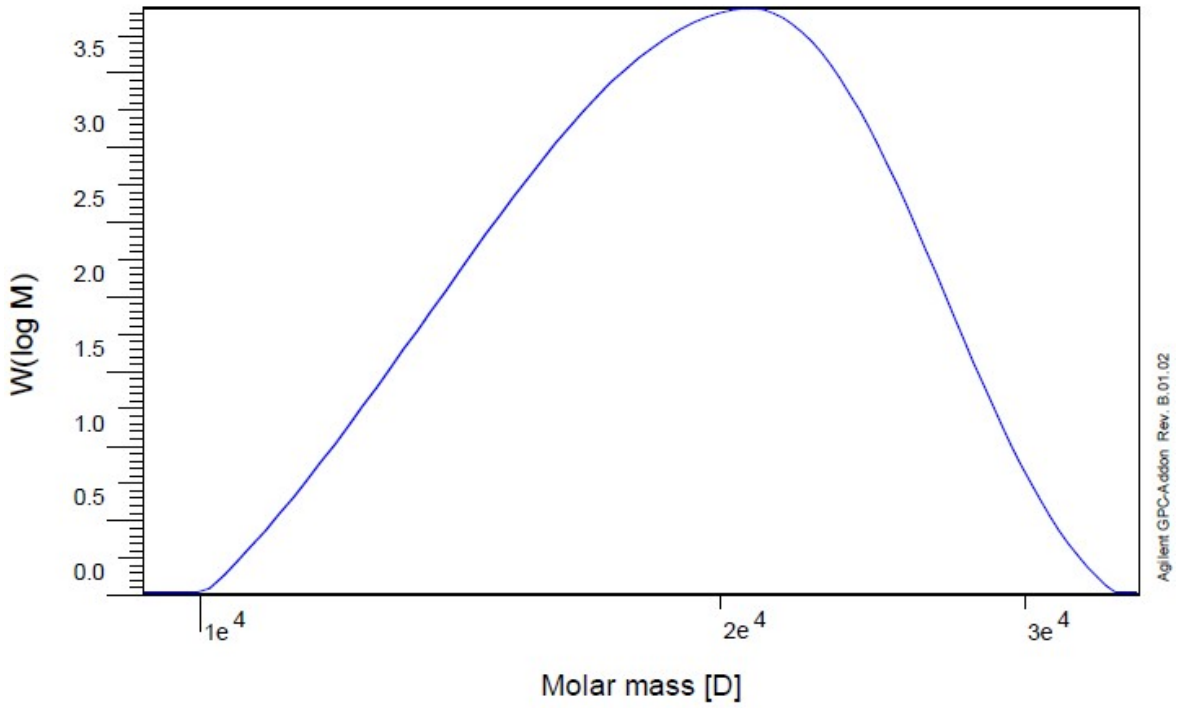


RID1A

Mn :	2.4305e4	g/mol
Mw :	3.2715e4	g/mol
Mz :	3.5825e4	g/mol
Mv :	0.000000	g/mol
D :	1.3045e0	
[n]:	0.000000	ml/g
Vp :	2.2666e1	ml
Mp :	2.9948e4	g/mol
A :	2.0961e3	ml*V
10%	1.4882e4	g/mol
30%	1.8922e4	g/mol
50%	2.2726e4	g/mol
70%	2.9521e4	g/mol
90%	3.5527e4	g/mol

GPC of PS1-PEG5000

Injection Date :	06-Aug-18, 16:06:23		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	22.429 min	**Baseline to :**	23.414 min
Integration from:	22.429 min	**Integration to :**	23.414 min
MHK - A (Cal.):	0.000000E+0	**MHK - K (Cal.):**	1.000000E+0 ml/g
Eluent :		**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	**Delay volume :**	0.000 ml
Operator :	SYSTEM	**Acquisition interval :**	0.430 sec

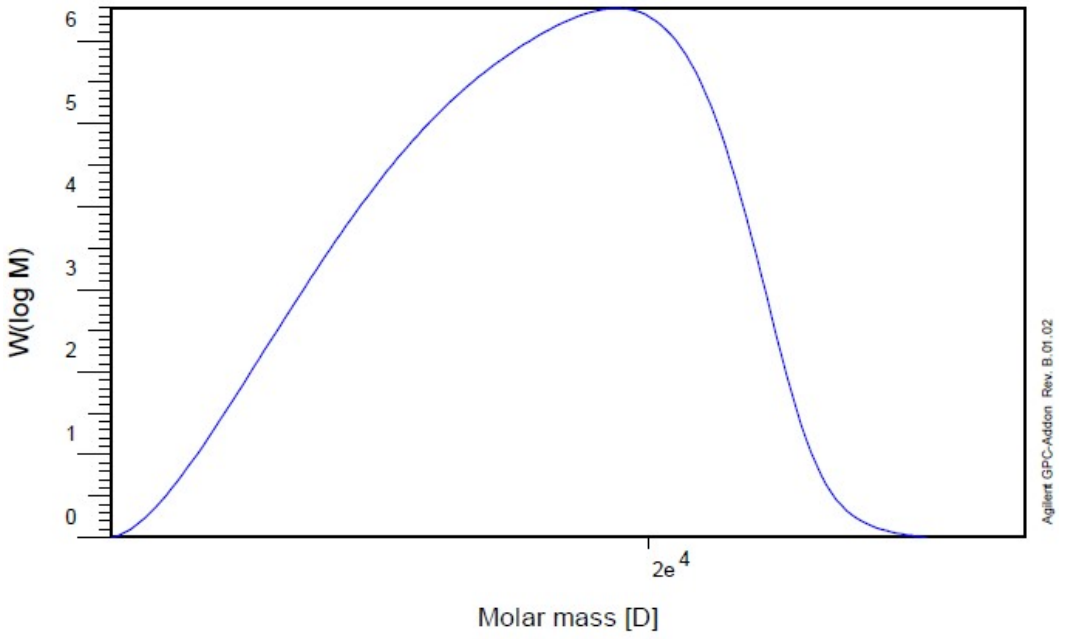


RID1A

Mn :	2.2184e4	g/mol
Mw :	2.7347e4	g/mol
Mz :	2.9410e4	g/mol
Mv :	0.000000	g/mol
D :	1.2327e0	
[n]:	0.000000	ml/g
Vp :	2.2947e1	ml
Mp :	2.4362e4	g/mol
A :	8.7427e2	ml*V
10%	1.3085e4	g/mol
30%	1.5782e4	g/mol
50%	1.9327e4	g/mol
70%	2.2882e4	g/mol
90%	2.8633e4	g/mol

GPC of PS2-PEG5000

Injection Date :	06-Aug-18, 18:51:20		
Calibration File :	E:\1METHOD\PS180712THF.CAL		
Calibration Date :	Friday 07/13/18 09:27:10		
Baseline from :	21.897 min	Baseline to :	23.059 min
Integration from:	21.897 min	Integration to :	23.059 min
MHK - A (Cal.):	0.000000E+0	MHK - K (Cal.):	1.000000E+0 ml/g
Eluent :		Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Detector 1 :	RID1A, Refractive Index Signal	Delay volume :	0.000 ml
Detector 2 :	VWD1A, Wavelength=450 nm	Delay volume :	0.000 ml
Operator :	SYSTEM	Acquisition interval :	0.430 sec



RID1A

$\overline{Mn}$:	1.9142e4	g/mol
$\overline{Mw}$:	2.3150e4	g/mol
$\overline{Mz}$:	2.7451e4	g/mol
$\overline{Mv}$:	0.000000	g/mol
D :	1.2093e0	
[n]:	0.000000	ml/g
Vp :	2.2349e1	ml
Mp :	2.2389e4	g/mol
A :	6.4363e3	ml*V
10%	1.5879e4	g/mol
30%	1.7689e4	g/mol
50%	1.9182e4	g/mol
70%	2.0635e4	g/mol
90%	2.4326e4	g/mol

GPC of PS3-PEG5000