Supporting Information

Synergistic Tailoring of Electrostatic and Hydrophobic Interactions for Rapid and Specific Recognition of Lysophosphatidic Acid, an Early-Stage Ovarian Cancer Biomarker

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Materials. Lysophosphatidic acid (LPA), glycerophosphoric acid (GPA), lysophosphatidylcholine (LPC), 10,12-pentacosadiynoic acid (PCDA) and 1-(3-Aminopropyl)imidazole were from Sigma-Aldrich. N,N'-Dicyclohexylcarbodiimide (DCC) and N-Hydroxysuccinimide (NHS) were purchased from Aladdin. Supel™-Select HLB SPE Tube (volume 3 mL, bed wt. 60 mg) were obtained from Sigma-Aldrich. All aqueous solutions throughout the experiments were prepared using ultra-pure water (18.2 MΩ, Milli-Q, Millipore).

Characterization. The ζ -potential of materials was collected on Malvern Zetasizer Nano ZS. The morphology of samples was obtained on a TECNAI G2 transmission electron microscope (TEM). UV-Vis absorption analysis was performed with a cary 50 scan UV-Vis-NIR spectrophotometer (Varian). Raman spectra were excitated using a 632 nm diode laser controller and collected on a LabRAM ARAMIS Raman spectroscope system (Scientific HORIBA) equipped with a charge-coupled device (CCD) detector.

Synthesis of imidazolium-functionalized diacetylene monomer. Firstly, NHS-modified PCDA was synthesized. NHS (300 mg), DCC (600 mg) and PCDA (0.750 g) were dispersed in CH_2Cl_2 (20 mL) by stirring at room temperature for under N_2 12 h. After white precipitate was filtered away, dichloromethane was evaporated, and the NHS-PCDA was obtained by flash column chromatography (eluant CH_2Cl_2, silica gel). Secondly, PCDA-NHS and 500 mg 1-(3-Aminopropyl)imidazole were dispersed in 10 mL CH_2Cl_2 by stirring for 24 h. After dichloromethane was evaporated, N-(3-(1H-imidazol-1-yl)propyl)pentacosa-10,12-diynamide was obtained by silica gel column (CH_2Cl_2/CH_3OH, 97:3). Finally, the obtained product (0.250 g) was dispersed in CH_3CN (20 mL) and then CH_3I (400 mg) was added. The mixed system was refluxed overnight. After the solvent was evaporated, imidazolium-functionalized diacetylene monomer was obtained.

The definition of colorimetric response (CR) of iPDAs to a given amount of LPA. To quantify the the response of iPDAs to LPA, the colorimetric response (CR) was calculated by the following equation:

$CR = [(B_0 – B_1)/B_0]*100$

where $B = I_{625} / (I_{625}+ I_{540})$, I is the intensity of absorption at 625 nm or 540 nm in the UV-vis spectrum. B_0 and B_1 are the percent blue before and after the color transition.

Extraction of LPA from plasma samples. Mouse or human whole blood in EDTA-containing tubes was centrifuged at 4000 rpm for 5 minutes. To remove remaining platelets, the supernatant was transferred to a microcentrifuge tube and centrifuged at 10000 rpm for 5 minutes. Then, the obtained human or mouse plasma (0.2 mL) was added into 2 mL MeOH–$CHCl_3$ ($V_{(MeOH)}$: $V_{(CHCl3)}$ = 2:1), and vortexed for 30 s. The mixed system was incubated for 20 min at 4℃. After the mixture was warmed to room temperature, it was centrifuged for 10 min at 2000 rpm. The obtained supernatant was transferred to 1 mL PBS (10mM, pH=7.4) and vortexed for 30 s at 2000 rpm. The upper aqueous solution containing LPA was obtained and then washed four times with 0.77 mL $CHCl_3$ to remove the neutral lipids. After that, the aqueous solution containing LPA is acidified to pH=2.0 with concentrated H_3PO_4 to protonate the LPAs. Afterwards, solid phase extraction (SPE) was used for the isolation and enrichment of the LPAs. The SPE cartridge was firstly preconditioned with methanol (6 mL) and H_2O (3mL). The acidified LPAs solution was then tranferred into the cartridge. Then, the cartridge was rinsed with H_2O (3 mL) followed by 6 mL $CHCl_3$. After the SPE cartridge was dried by N_2, LPA was eluted with MeOH (2 mL). After evaporation of MeOH by N_2 stream, LPAs was dispersed in 0.1 mL H_2O.The LPA recovery rate was about 51%.

Molecular Dynamic Simulations.

Molecular dynamics simulations (MD simulations) were used to calculate interaction energy after iPDAs interacted with LPA. All simulations were performed with COMPASS force field, in the NVT ensemble at a temperature of 298K for 200ps with

a time step of 1fs.The density of the simulation system is 1.0 g/cm^3. The periodic boundary condition was added in all three directions. In the model of HE type, the length of the simulation box is 45.57Ångstrom. But in the model of H type, the length of box (62.50 Ångstrom) is slightly larger than that of HE type in order to keep enough distance between LPA and their images.

Density functional theory (DFT).

DFT calculations were performed using B3LYP functional with the 6-31G(d) basis set in Gaussian09 program. The geometry of each compound was first relaxed by molecular dynamics simulations. Then, the backbone of iPDAs was selected to perform the DFT calculations. In this way, we can focus onthe change of LUMO-HOMO gap of backbone after iPDAs interact with LPA.

Tumor models.

Animal handing procedures were performed in accordance with the guidelines of the Regional Ethics Committee for Animal Experiments. Female C57BL/6J mice were implanted subcutaneously or intraperitoneally with ID8 cells. Female Balb/c mice were implanted subcutaneously or intraperitoneally with 4T1 cells.

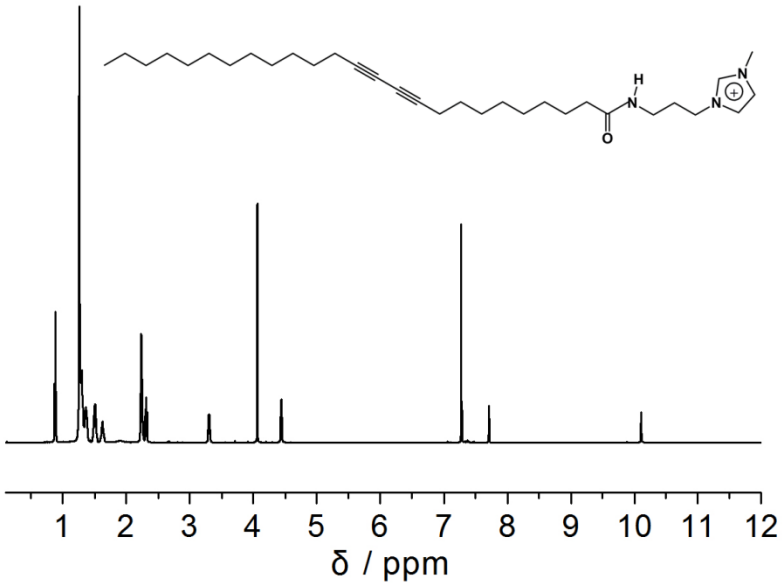

Figure S1: ^{1}H NMR of imidazolium-functionalized diacetylene monomer in $CDCl_3$.

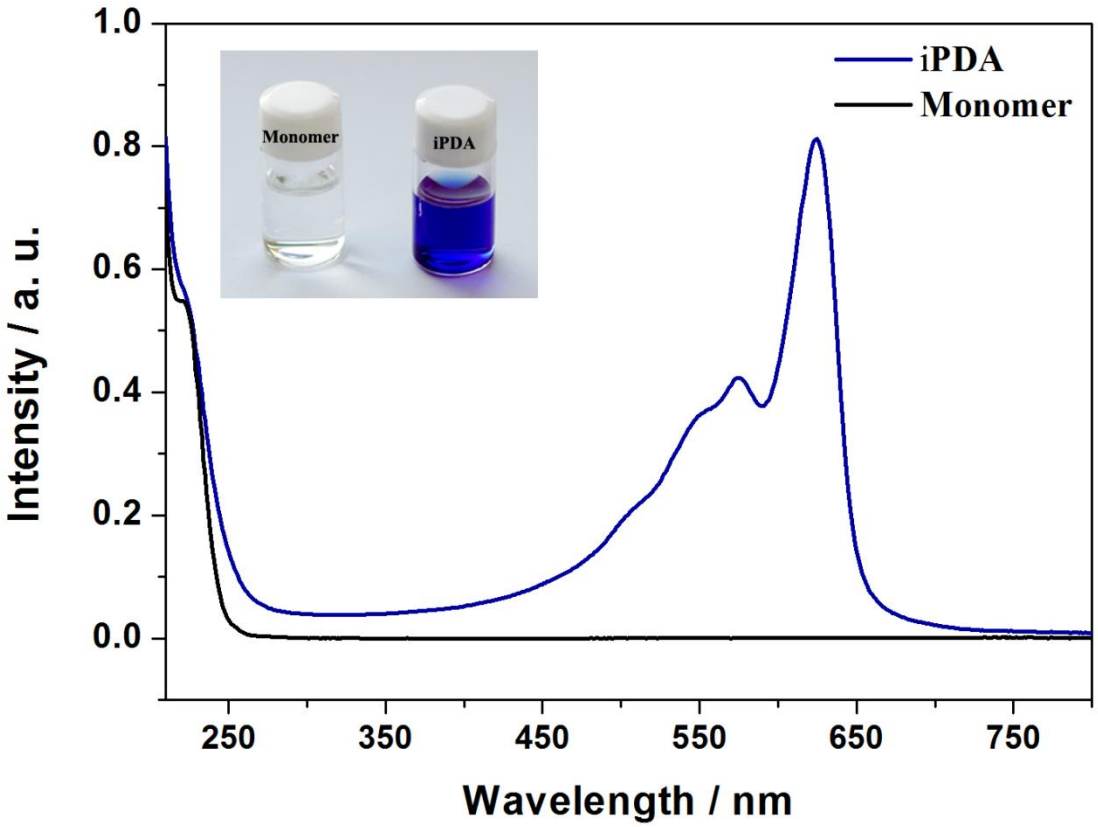

Figure S2: Visible absorption spectral of monomer (black line) and iPDAs (blue line), respectively. Inset is photograph of monomer and iPDAs in HEPES buffer.

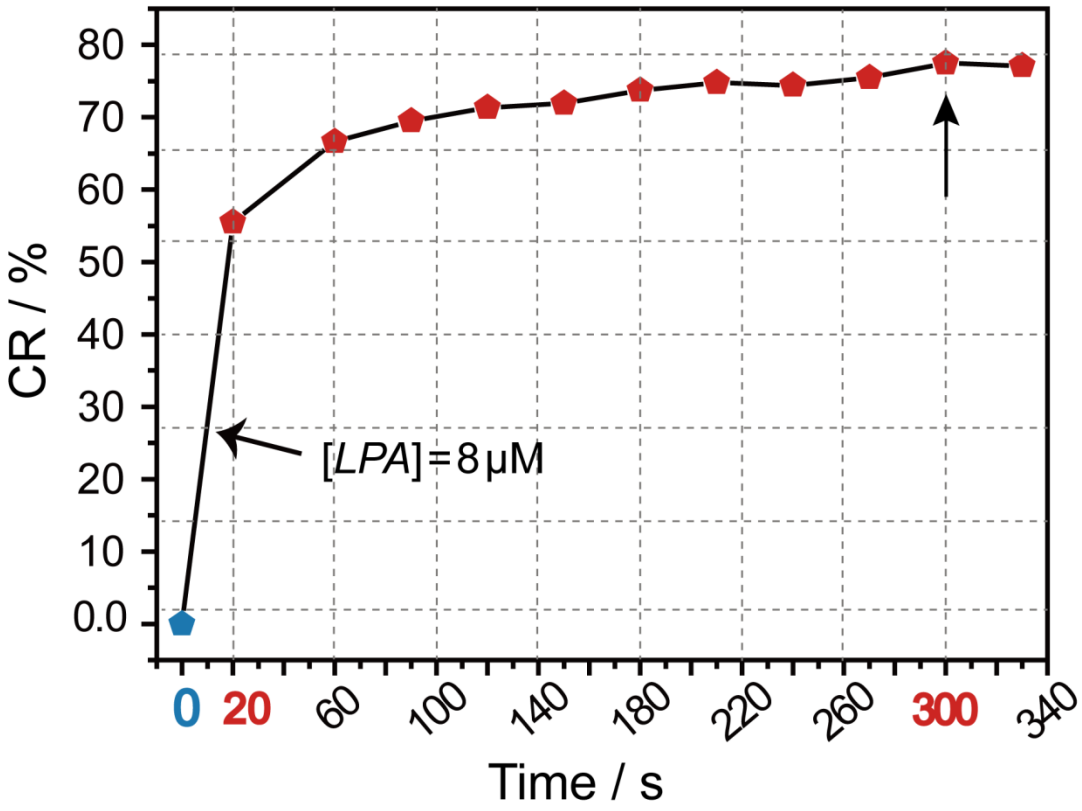

Figure S3: CR of the iPDAs toward 8μM LPA at different time points.

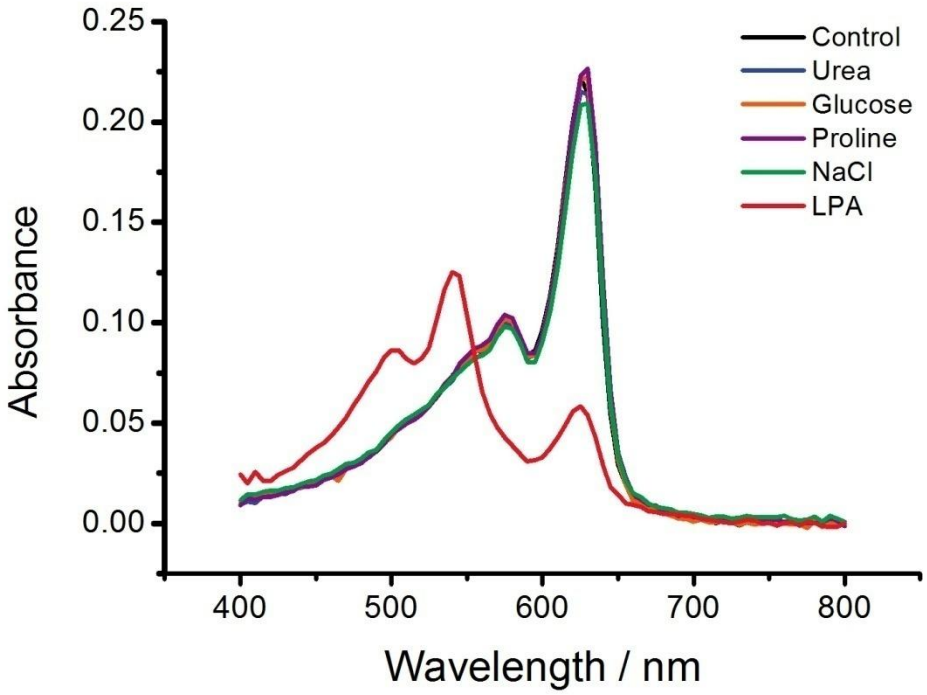

Figure S4: Visible absorption spectral changes of iPDAs in the presence of urea, glucose, proline, NaCl and LPA.

Figure S5: Photograph of iPDAs in the presence of urea, glucose, proline, NaCl and LPA.

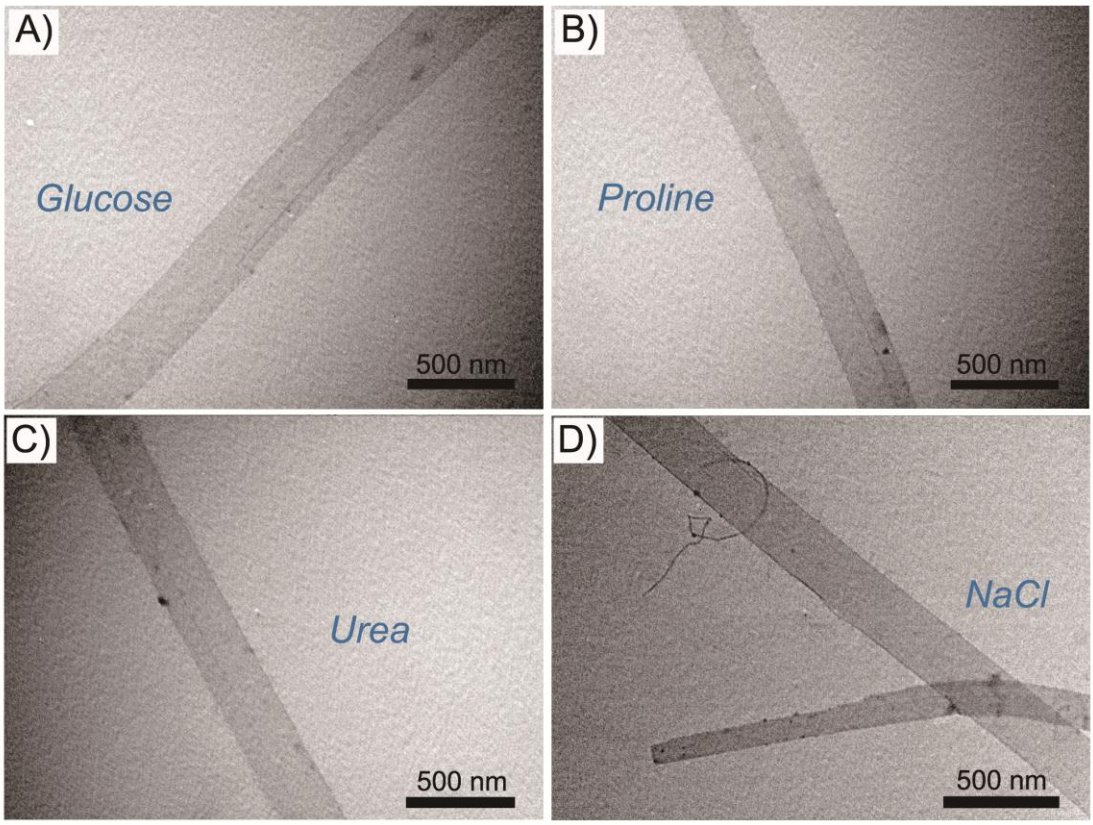

Figure S6: TEM images of iPDAs in the presence of urea, glucose, proline and NaCl.

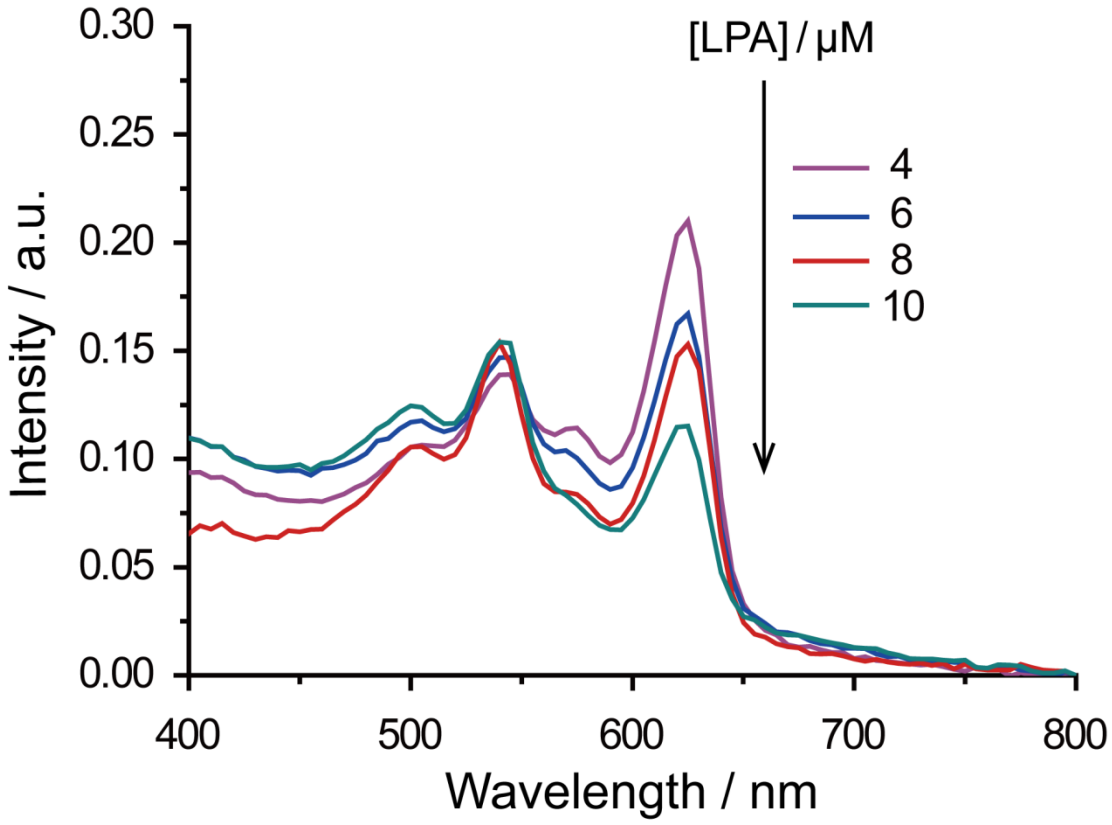

Figure S7: Visible absorption spectral changes of iPDAs in the presence of different LPA concertrations extracted from plasma.

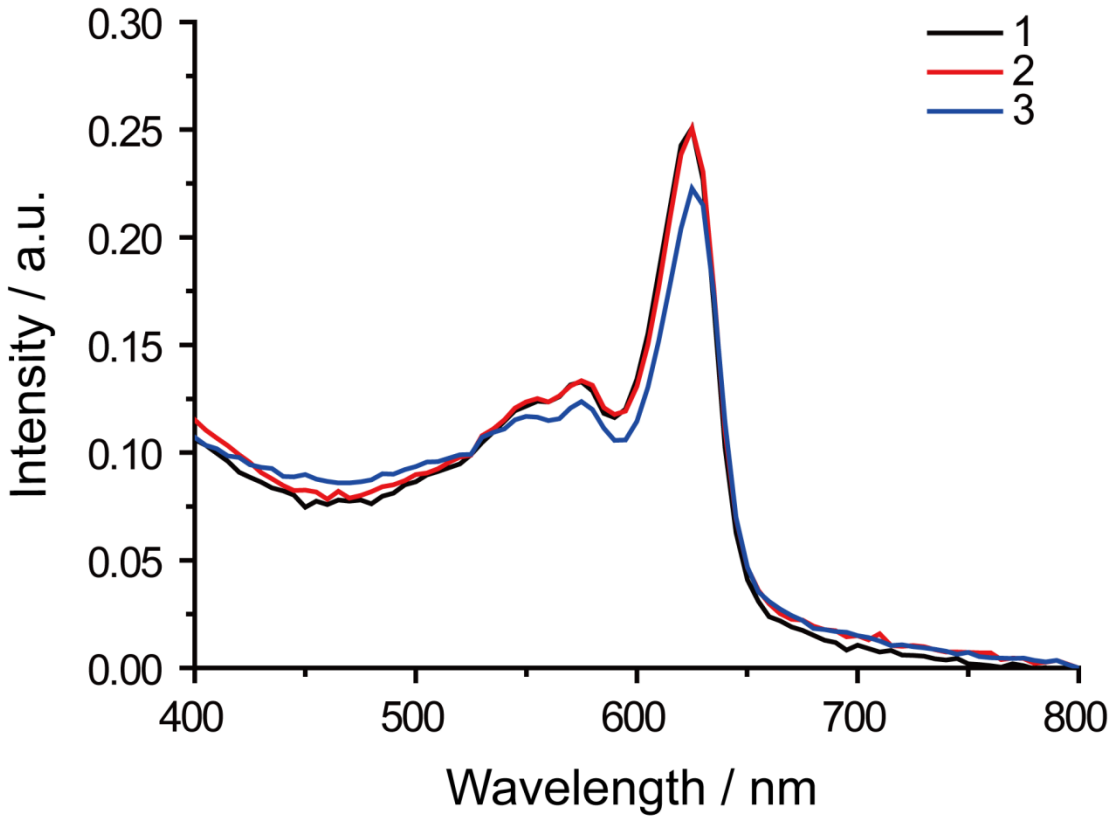

Figure S8: Visible absorption spectral changes of iPDAs in the presence of extracted LPA from healthy C57BL/6 mice.

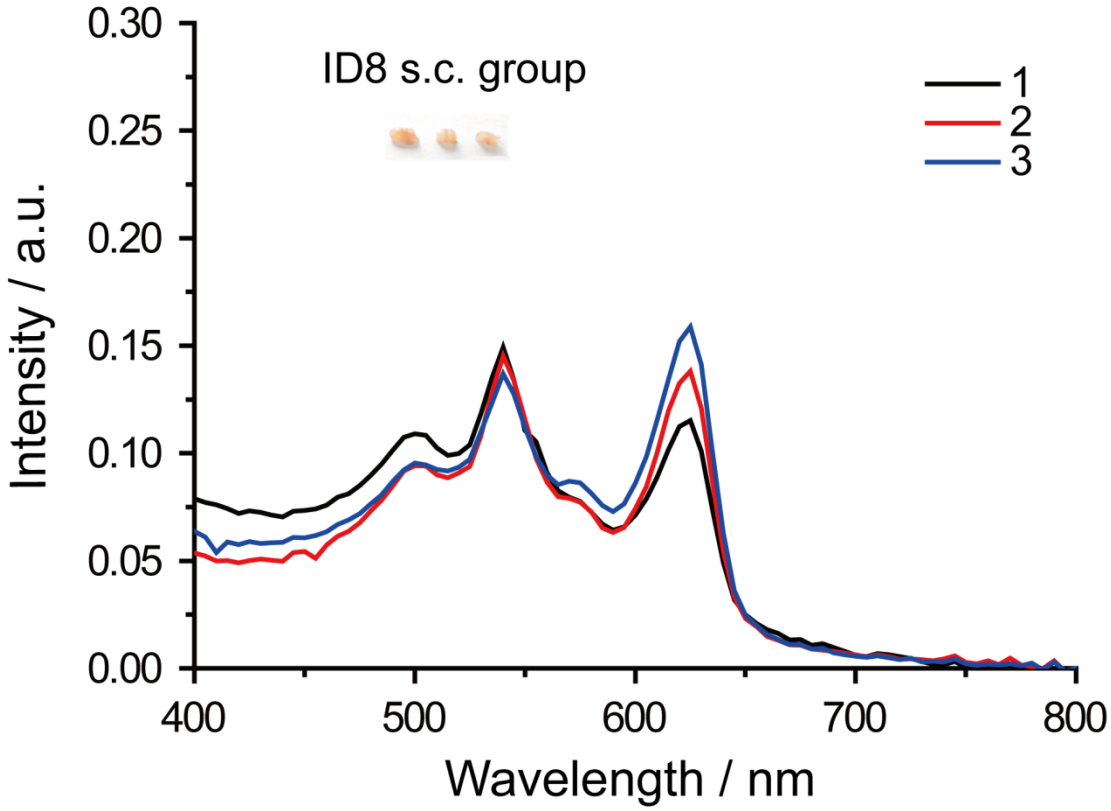

Figure S9: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing small subcutaneous (s.c.) ovarian (ID8) tumor.

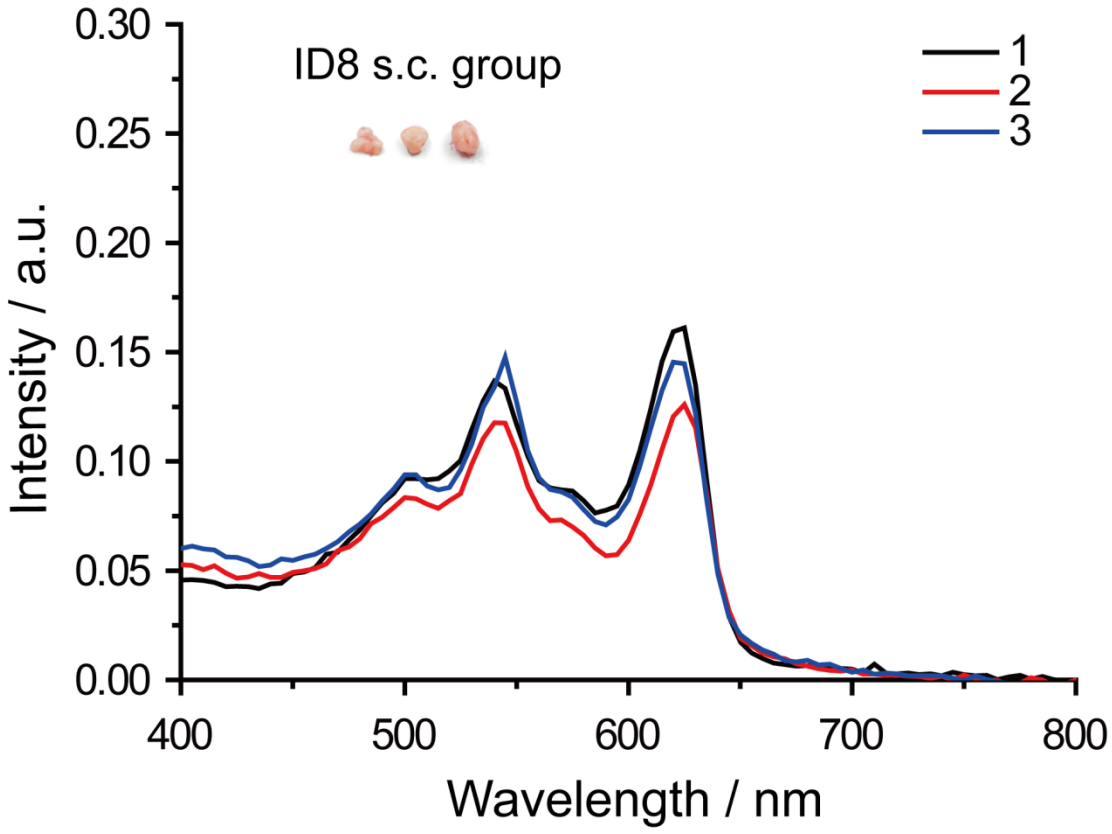

Figure S10: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing big subcutaneous (s.c.) ovarian (ID8) tumor.

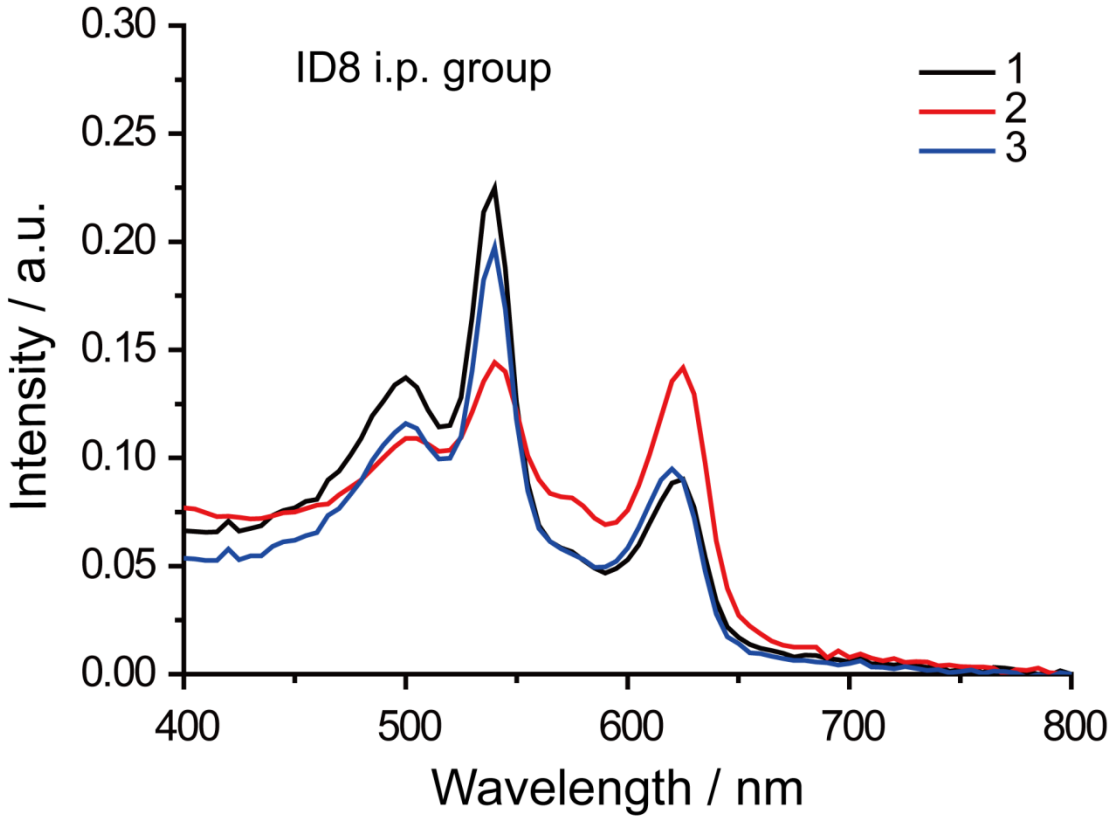

Figure S11: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing metastatic tumor model developed by intraperitoneal (i.p.) inoculation of ID8 tumor cells.

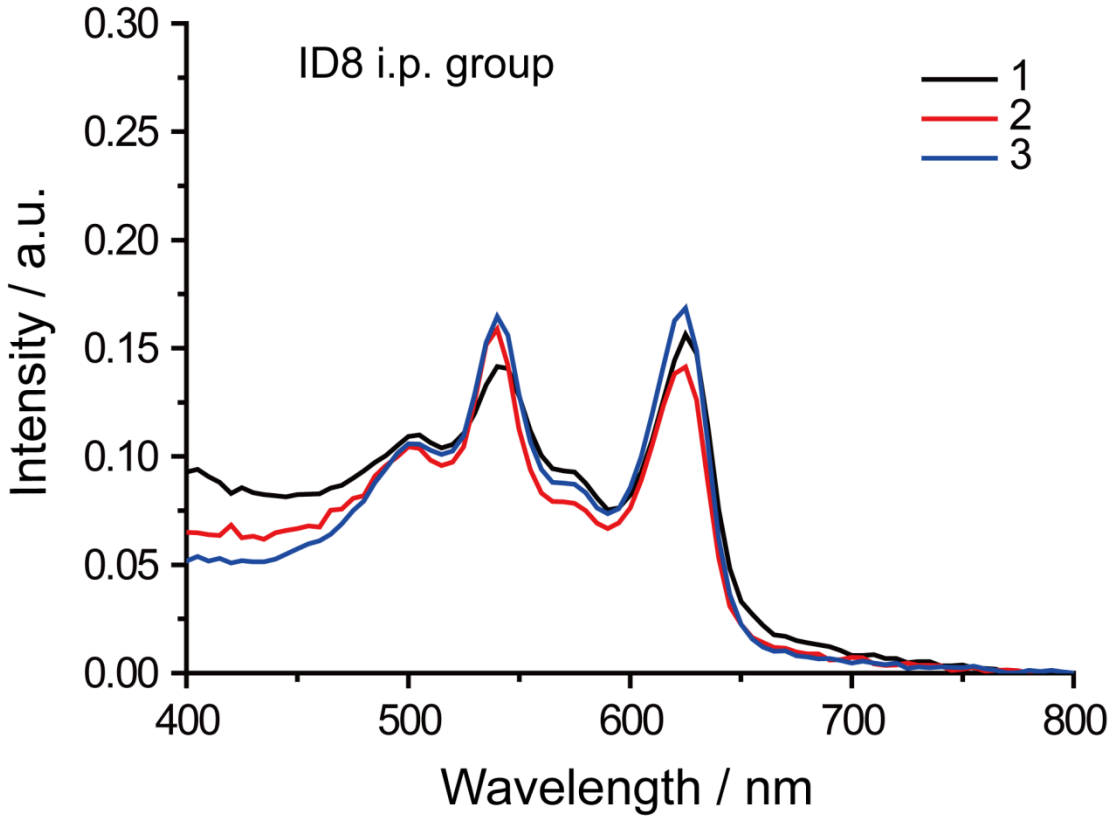

Figure S12: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing metastatic tumor model developed by intraperitoneal (i.p.) inoculation of ID8 tumor cells.

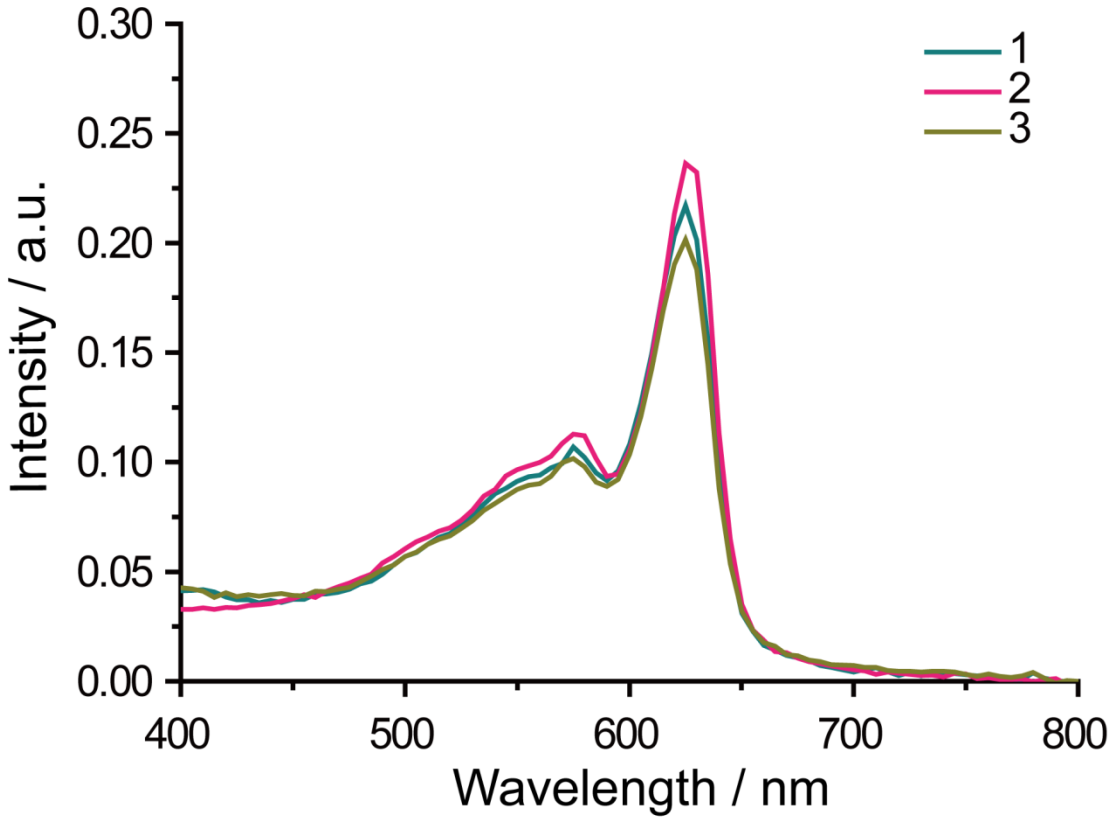

Figure S13: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from healthy Balb/c mice.

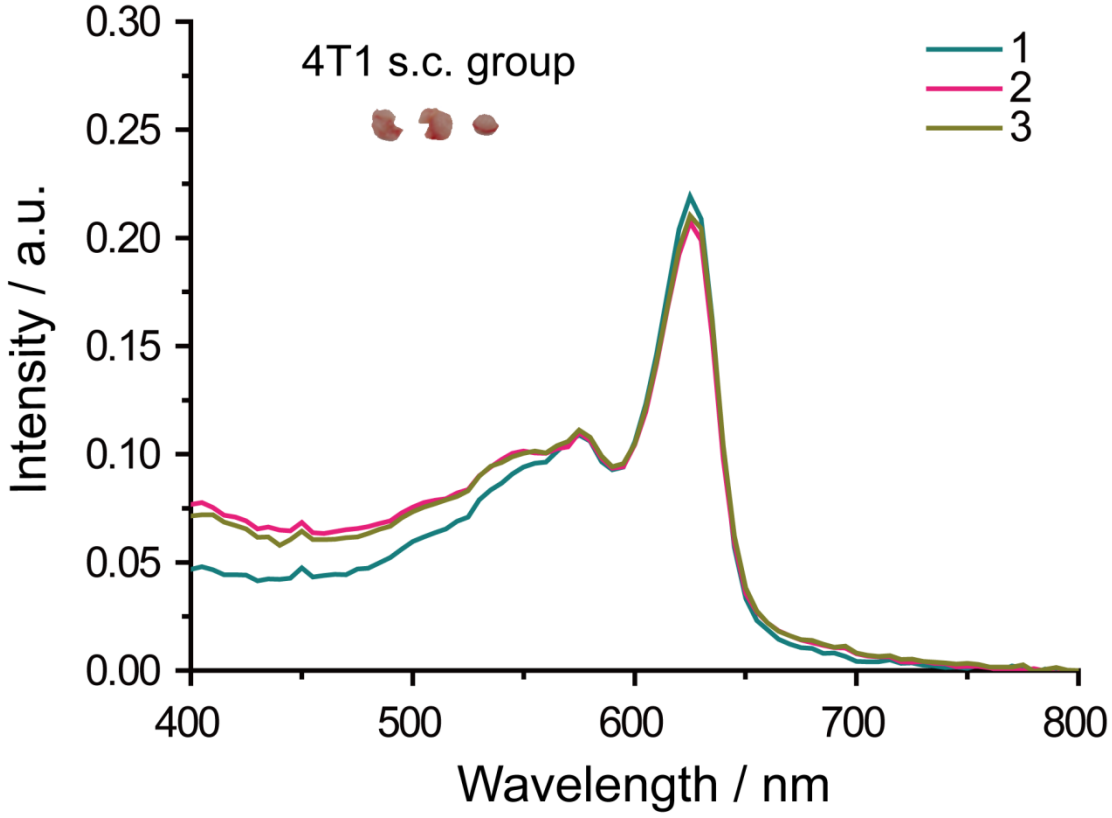

Figure S14: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing small subcutaneous (s.c.) breast (4T1) tumor.

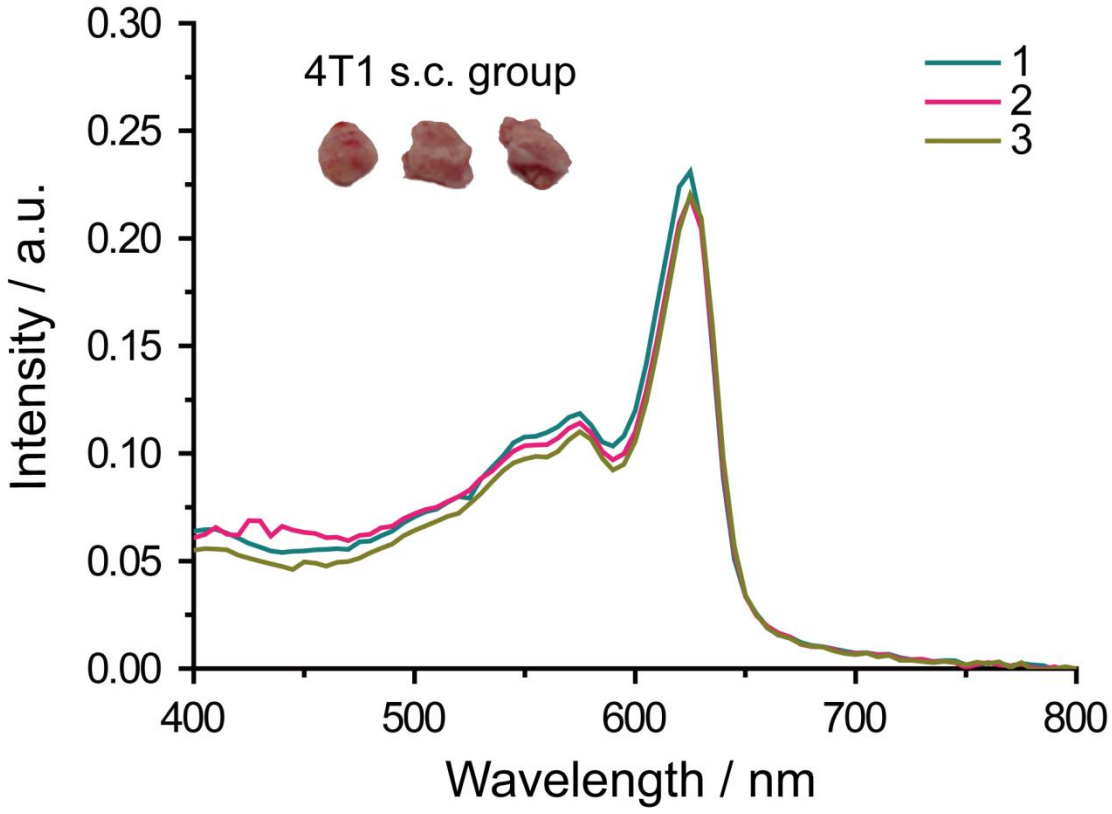

Figure S15: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing big subcutaneous (s.c.) breast (4T1) tumor.

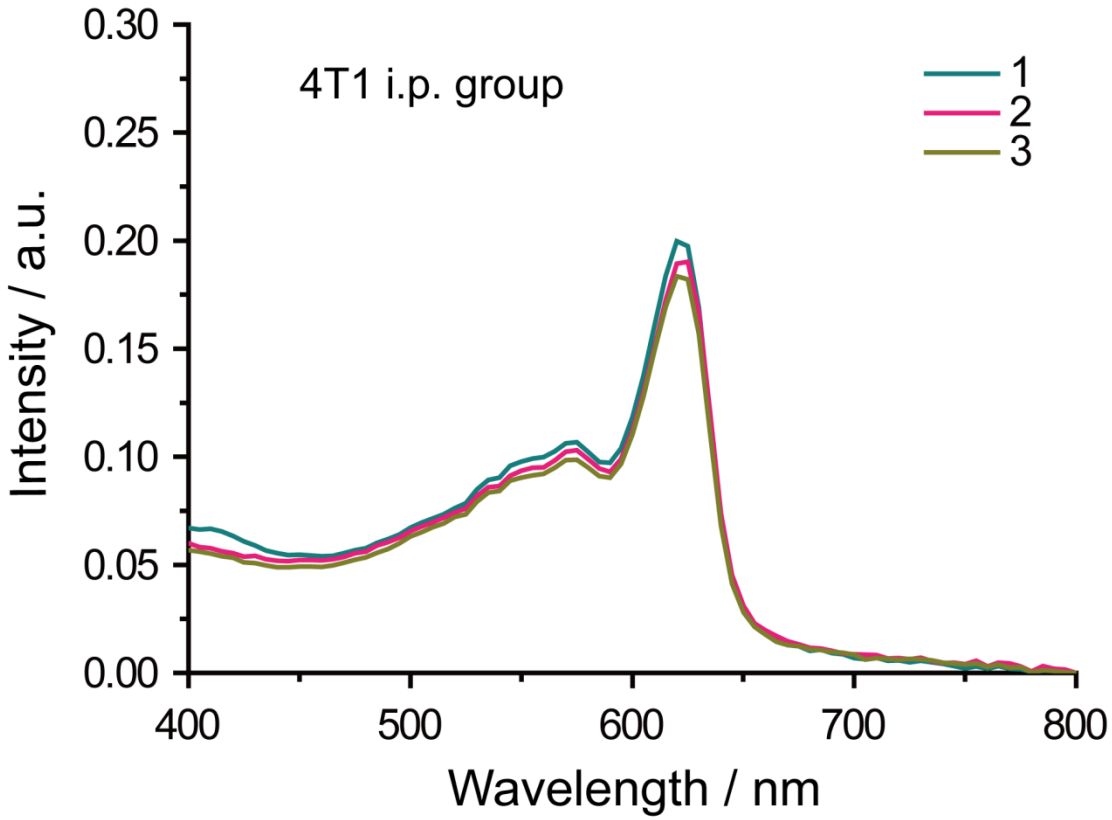

Figure S16: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing metastatic tumor model developed by intraperitoneal (i.p.) inoculation of 4T1 tumor cells.

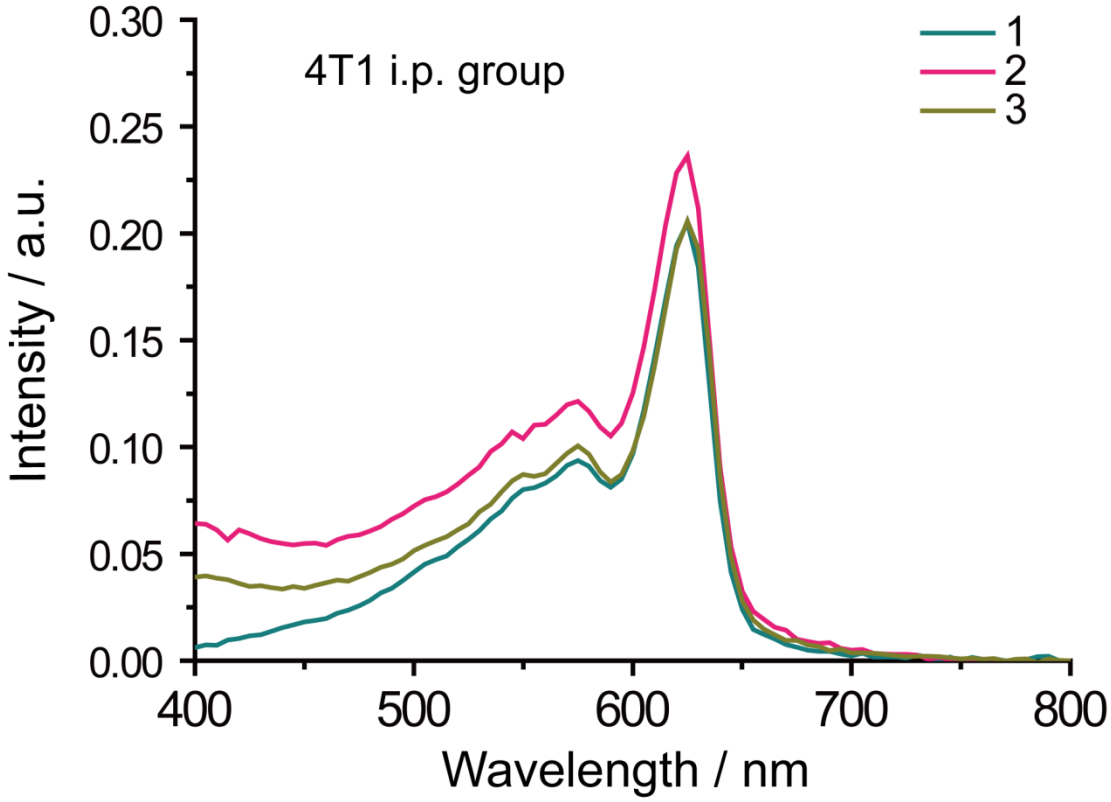

Figure S17: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from mice bearing metastatic tumor model developed by intraperitoneal (i.p.) inoculation of 4T1 tumor cells.

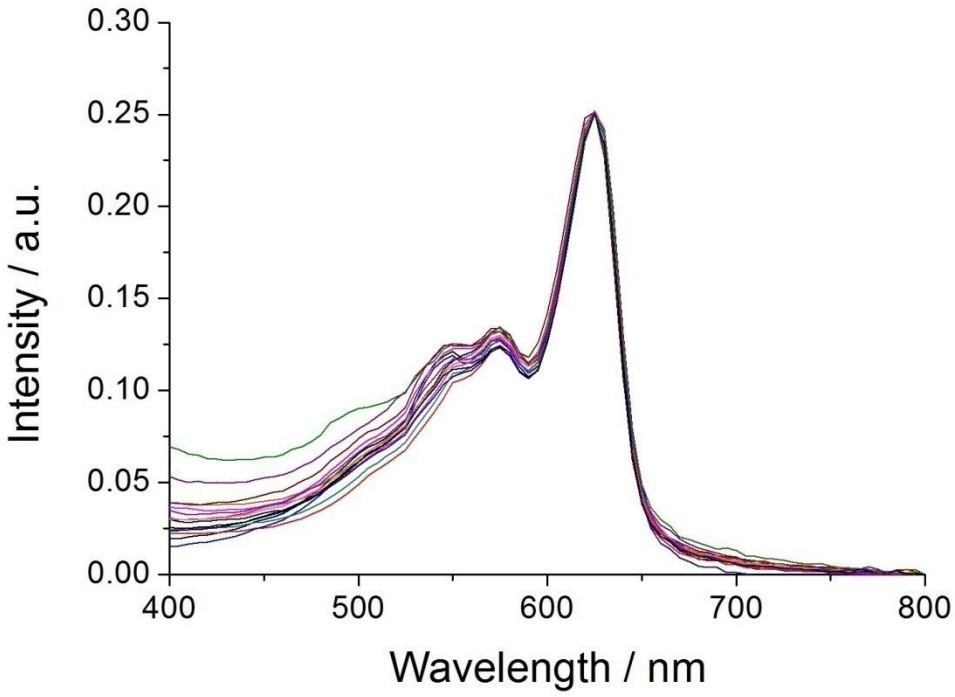

Figure S18: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from healthy volunteers.

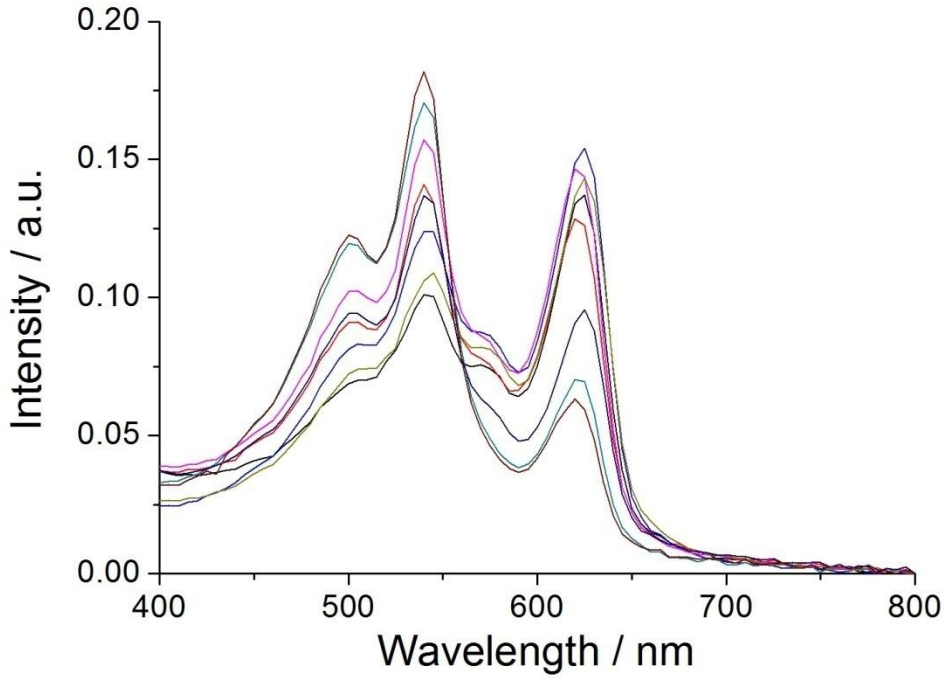

Figure S19: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from ovarian cancer patients at stage I.

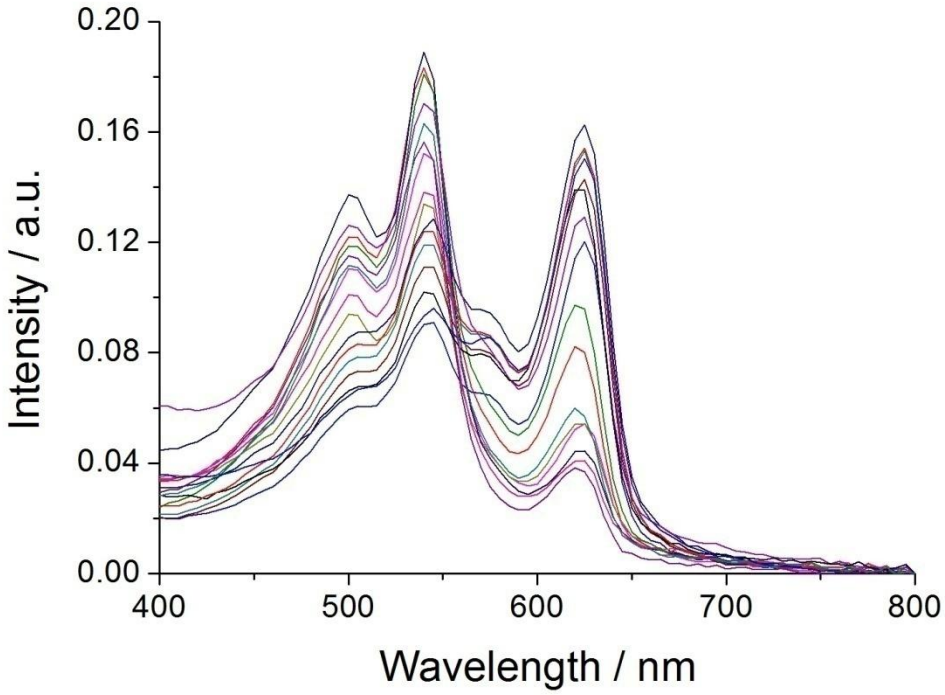

Figure S20: Visible absorption spectral changes of the iPDAs in the presence of extracted LPA from ovarian cancer patients at stage II-IV.

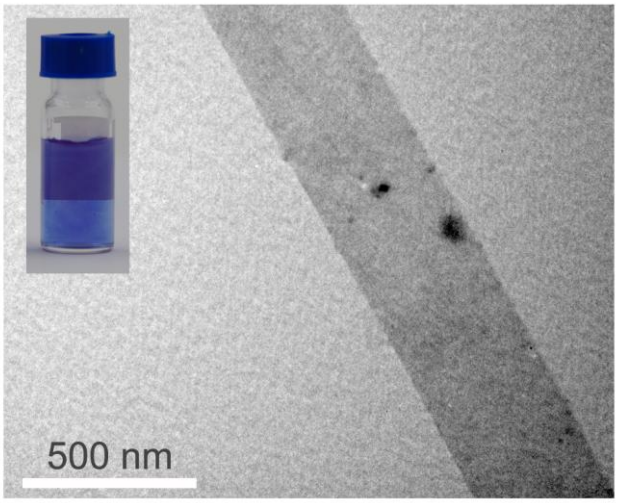

Figure S21: TEM imaging of iPDAs preserved in HEPES buffer for 2 months. The inset showed the photograph of iPDAs preserved in HEPES buffer for 2 months.

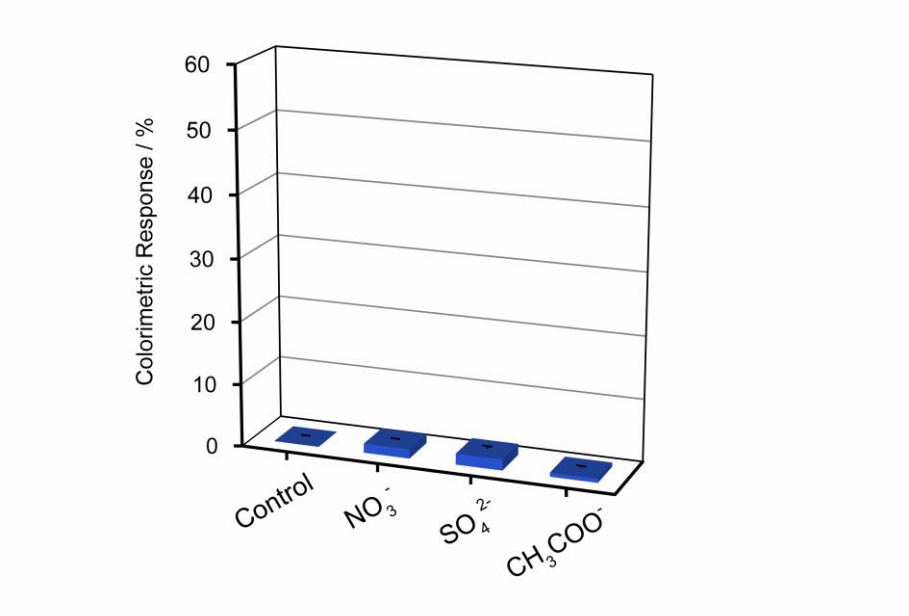

Figure S22: CR of the iPDAs after addition of sulfate, acetate and nitrate.

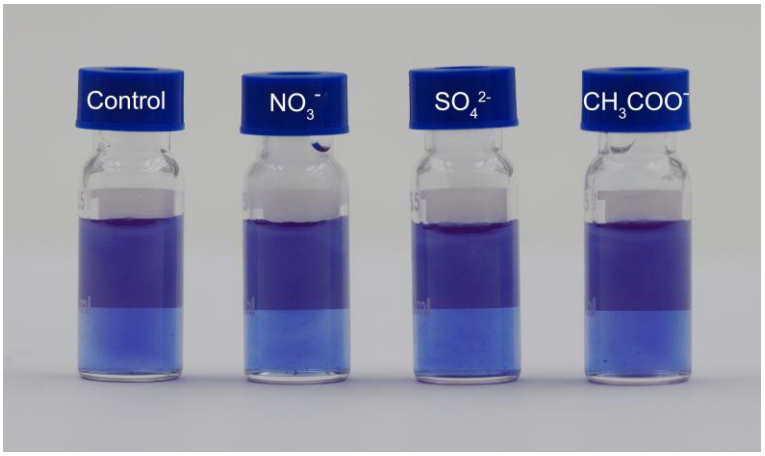

Figure S23: Photograph of iPDAs after addition of sulfate, acetate and nitrate.

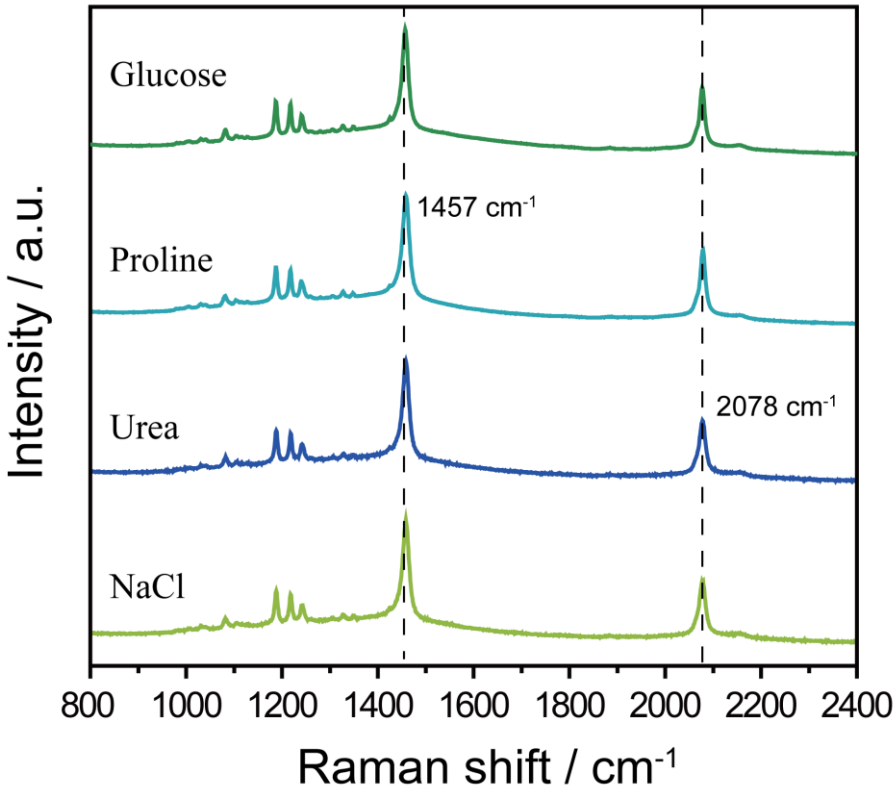

Figure S24: Raman spectra of iPDAs in the presence of glucose, proline, urea and NaCl

Table S1: Recoveries of LPA species from plasma after SPE enrichment.

LPA Concentration added / µM	LPA Concentration extracted / µM	Recovery / %		STDEV
4.0	1.8	45	51	0.71
6.0	3.4	57		
8.0	4.1	51		
10.0	5.2	52		