Ultrastable Core-Shell structured Nanoparticles Directly Made from Zwitterionic Polymers

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Supporting Information

Materials

Methacryloyloxyethyl phosphorylcholine (MPC 97%), [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA 97%), Tert-Butyl bromoacetate (98%), 2-(Dimethylamino) ethyl mathacrylate (DMA 98%), Amberlite IRN78 hydroxide form, Quinine hemisulfate salt monohydrate (98%), Acetone (99.5%), Phosphate buttered Saline tablet (PBS), Albumin from bovine serum (BSA 98%) and Fibrinogen from bovine plasma (65-85%) were purchased from Sigma-Aldrich, St. Louis, MO. Ethyl Ether Anhydrous (99.9%) and Boric Anhydride were obtained from Fisher Chemical. Co. Acetonitrile anhydrous (99.9%) was purchased from ACROS Organics. B-Propiolactone (95%) was supplied by Wako Pure Chemical Industries Ltd. Deuterium Oxide (D2O 99.9%) was obtained from Cambridge Isotope Laboratories, Inc. USA. PD-10 desalt columns were purchased from GE Healthcare, UK. Boric Anhydride was utilized to dry acetone and all other chemicals were used without further purification.

Synthesis of PSB NPs, PMPC NPs, PCB-2 NPs

5 mg photo-initiator was added to 5 ml degassed DI water and 100 mg SBMA, MPC, CBMA-2 (synthesis as previous report) monomer was dissolved 0.5 ml photo-initiator solution. Polymerization was initiated by exposing the solution under UV (365 nm, 6 W) for 2 minutes with stirring. Then resulted polymer solution was diluted to 2.5 ml and went through salt filter column (cutoff molecular weight 2000) to remove residue monomer and initiator. Pure polymer (Mw 10k) solution was poured into a flask and the flask was set in the center of a domestic microwave oven. After heating under 1200 W for 5 minutes, light brown substance emerged in the bottom of the flask. 10 ml DI water was added and stirred for 1 h. Resulting solution was centrifuged at 4400 rpm for 10 min to remove the insoluble, yielding a clear light brown NPs solution with florescence

under UV. The NP yeild was 56.65%.

Synthesis of PCB-1 NPs

CBMA t-butyl monomer was synthesized as previous report1. 200 mg of CBMA tbul monomer was hydrolyzed in 2 ml TFA for 5h. TFA solution was dropped in anhydrous ethyl ether to remove TFA and precipitate CBMA-1 zwitterionic monomer. After vacuum dried, monomer was dissolved in 0.5 ml photo-initiator solution described above in ice bath. Basic resin was used to adjust solution pH to 7 and the yielding solution was exposed under UV to initiate polymerization. The resulting PCB-1 was similarly purified and made into NPs as described in the last section.

NPs purification

2 g sucrose,was dissolved in 10 ml DI water to prepare sucrose stock solution. . 1.4 ml sucrose solution was added to a centrifuge tube and 0.1 ml NPs raw solution was slowly added to obtain a well-defined NP region on the top of sucrose solution. Centrifuge the tube for 1 h at 15000 rpm at room temperature and expose the tube to UV. NP region in the sucrose column showed blue florescence and was collected using a pipette. The collected solution will go through a PD-10 column to remove contaminated sucrose.

Characterization:

The morphology and microstructure of the PMPC NPs and PCB-1 NPs were examined by transmission electron microscopy (TEM) on JEOL 2010 TEM with LaB6 Filament Gun (JEOL Ltd. Tokyo, Japan) under accelerating voltage of 200 kV. Purified sample solutions were dropped onto a 300-mesh copper grid coated with a lacy carbon film to make TEM sample. Nuclear Magnetic Resonance H1 spectrum of zwitterionic polymer and purified NPs were conducted on Varian Mercury-400 MHz NMR. Polymer and purified NPs were lyophilized and dissolved in Deuterium Oxide (D2O). Molecular weight of the MPC and CB-1 polymer are determined by a Waters Alliance 2695 Separations Module equipped with a Waters Ultrahydrogel Linear column and a Waters 2414 reflex detector. The mobile phase was PBS buffer solution at a flow rate of 0.7 ml/min at 35 oC. Poly(ethylene oxide) from Polymer Laboratories were used as standards. UV absorption of NPs was conducted on a Multiskan GO UV–Vis Spectrophotometer (Thermo Scientific, USA).

Fluorescence Property measurement

The maximum absorptions were 354 nm for PMPC NPs and 367 nm for PCB-1 NPs. Maximum emissions are 436 nm for PMPC NPs and 455 nm for PCB-1 NPs. Quantum yield of the PMPC NPs and PCB-1 NPs were determined by comparative method. Quinine hemisulfate in 0.1 M

H2SO4 was utilized as the standard whose QY was reported to be 54%. The integrated fluorescence intensity is the area under the PL curve in the wavelength range from 380 to 650 nm with 365 nm excitation. Absolute values were calculated according to the following equation:

$$QY_{CDs} = QY_{ST} * \frac{Grad_{CDs}}{Grad_{ST}} * \frac{\eta^2_{CDs}}{\eta^2_{ST}}$$

Where ST denotes the standard, Grad is the gradient from the plot of integrated fluorescence intensity vs absorbance, and η is the refractive index of the solvent. To prevent the re-absorption effect, absorbance in the 10 mm fluorescence cuvette should never exceed 0.1 at the excitation wavelength. The resulted QY for PMPC NPs and PCB-1 NPs were 8.92±0.60 % and 7.02±0.59 %, respectively.

MTT cytotoxicity assays

The cytotoxicity of the PMPC NPs and PCB-1 NPs was determined by MTT assay. NIH/3T3 Fibroblast were grown in 96-well plates in full Dulbecco's Modified Eagle's Medium and 10% FBS under 5% CO2 at 37 oC to allow 80-90% confluence. For each well, cells were washed with PBS and incubated with 200 µl full medium containing varied concentration of PMPC NPs, PCB-1 NPs and CA NPs for 24 h. Cells were washed with PBS to remove NPs and incubated with 100 µl full medium plus 50 µl of 12 mM MTT stock solution for another 4 h. Then, MTT containing medium was replaced with 150 µl DMSO to dissolve the formed crystal at 37 OC for 10 min. Absorbance (Abs) was measured at 570 nm with pure DMSO as the blank reading. Cells with no NPs incubation were used as the controls and cell viability upon NPs treatment was estimated in triplicate: cell viability (%) = Abssample / Abscontrol x 100.

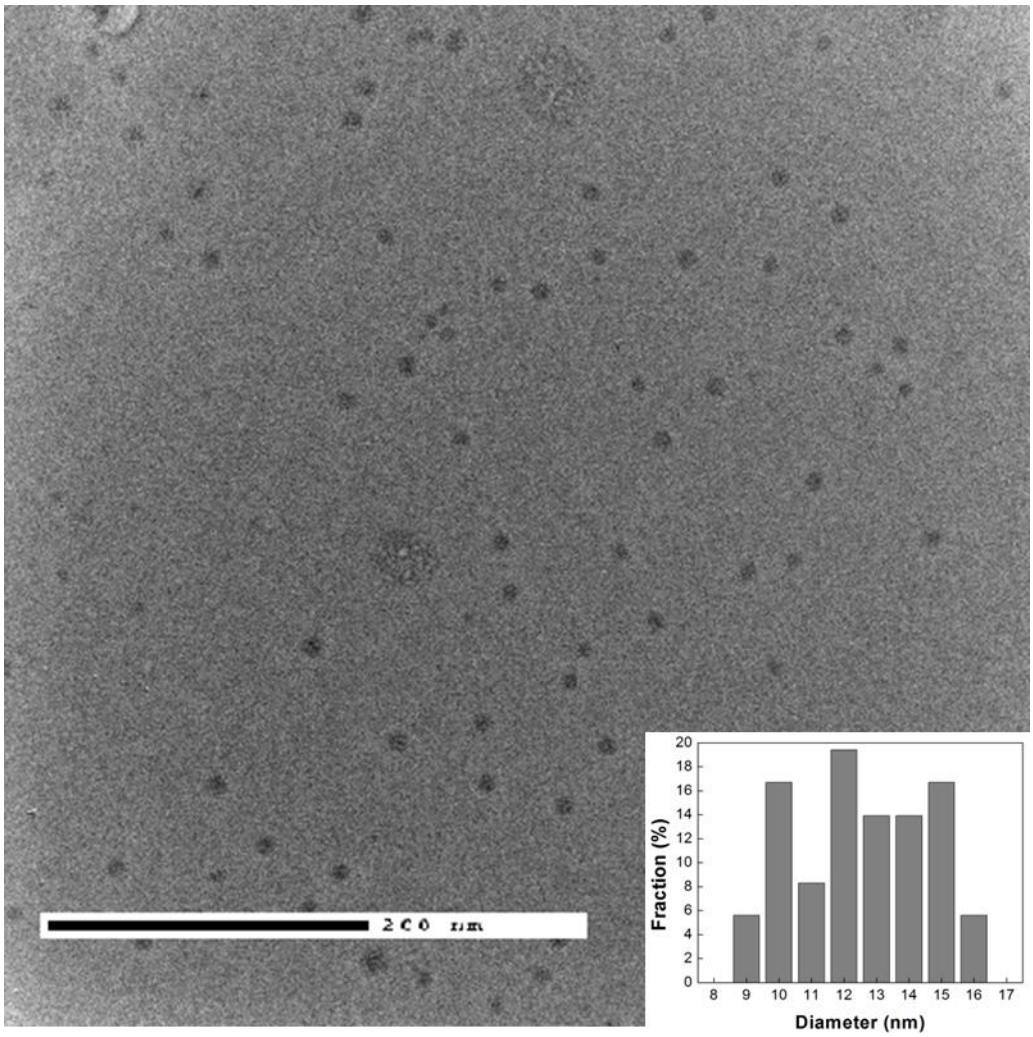

Fig. S1 TEM image of PMPC NPs

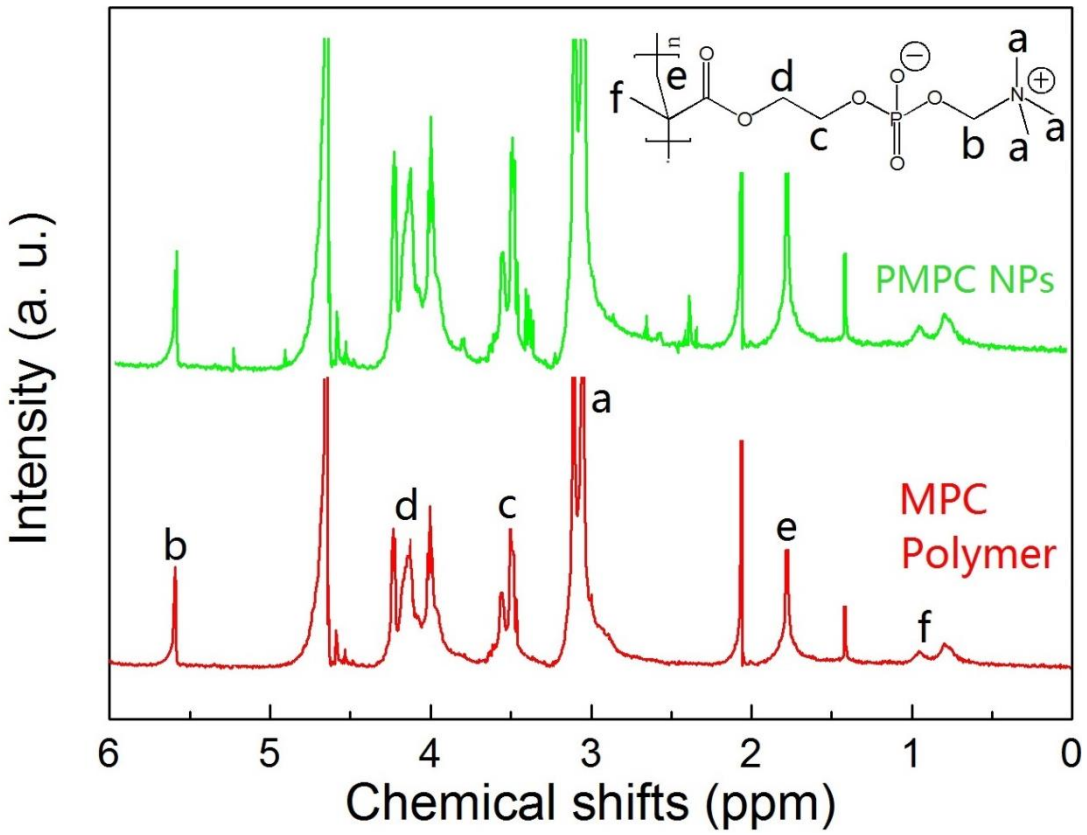

Fig. S2 NMR spectra of MPC Polymer and PMPC NPs

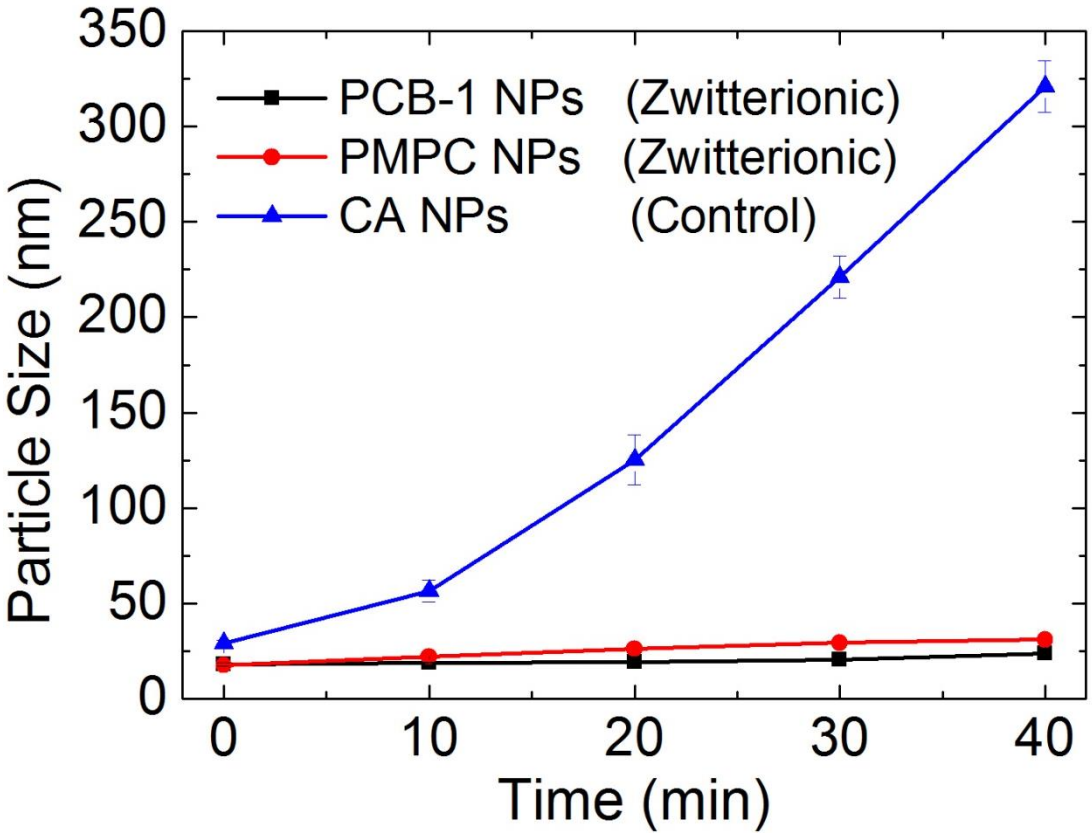

Fig. S3 Colloid stability of PCB-1, PMPC, and CA NPs in PBS buffer

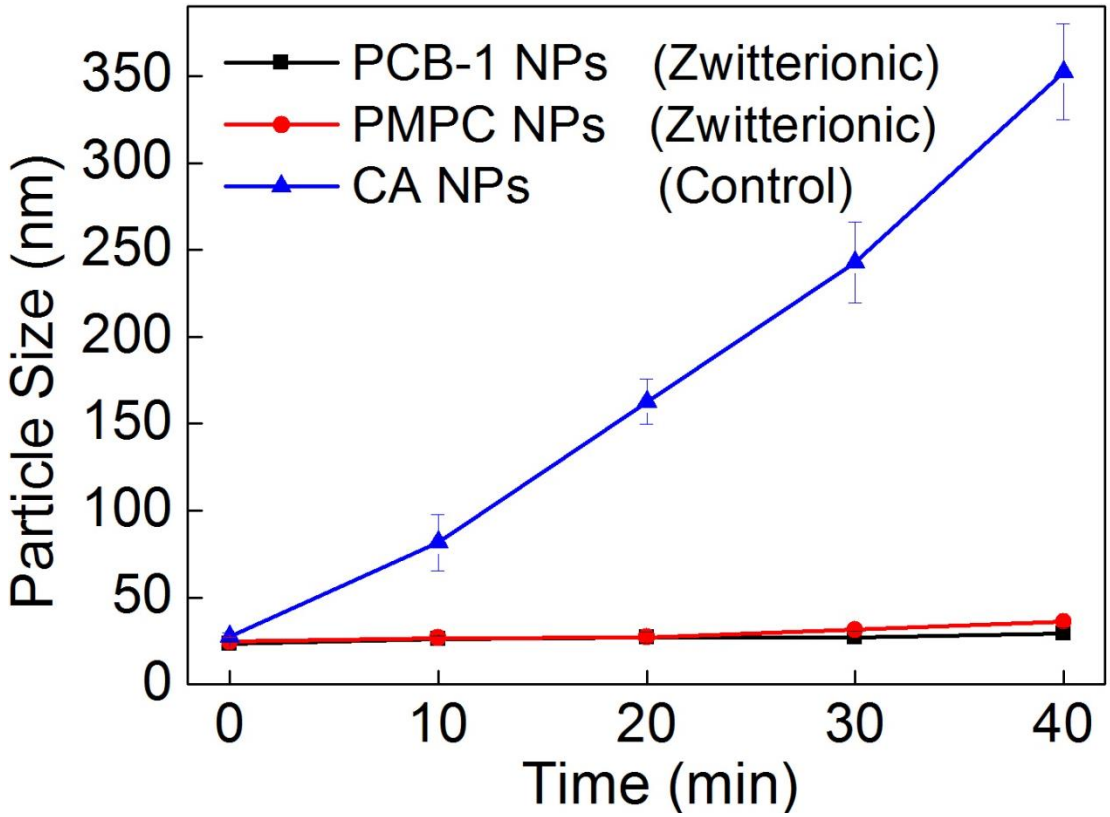

Fig. S4 Colloid stability of PCB-1, PMPC and CA NPs in fibrinogen solution in PBS buffer

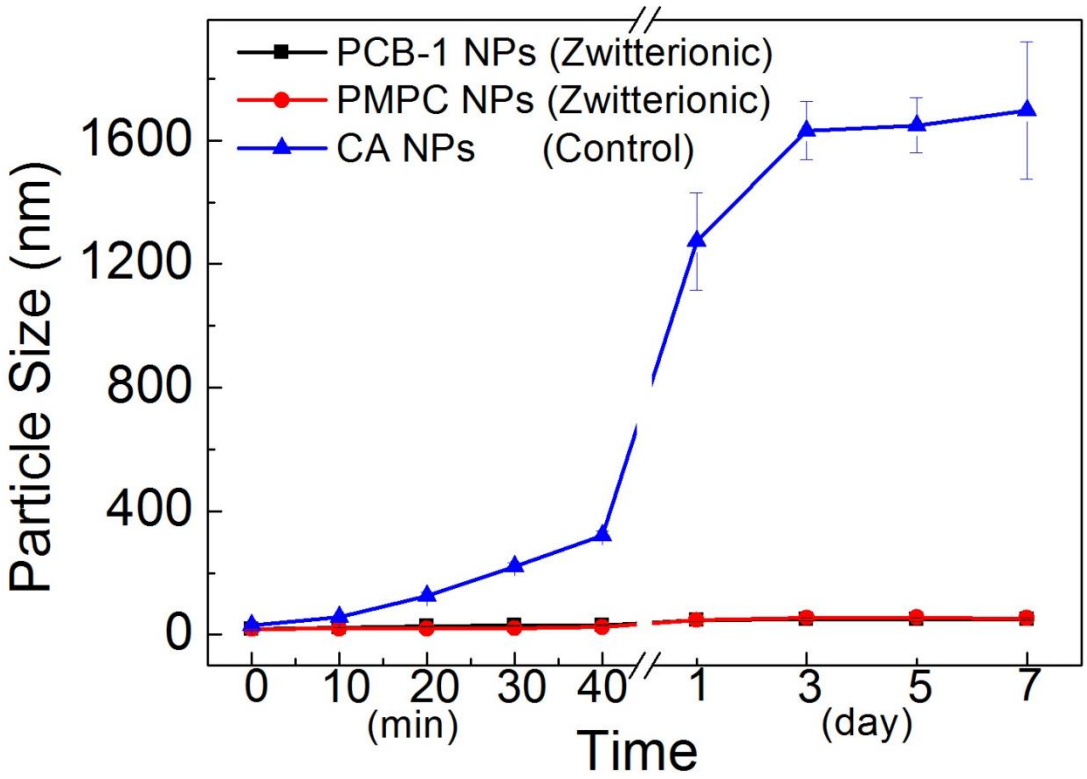

Fig. S5 Long-term stability of PCB-1, PMPC, and CA NPs in PBS buffer

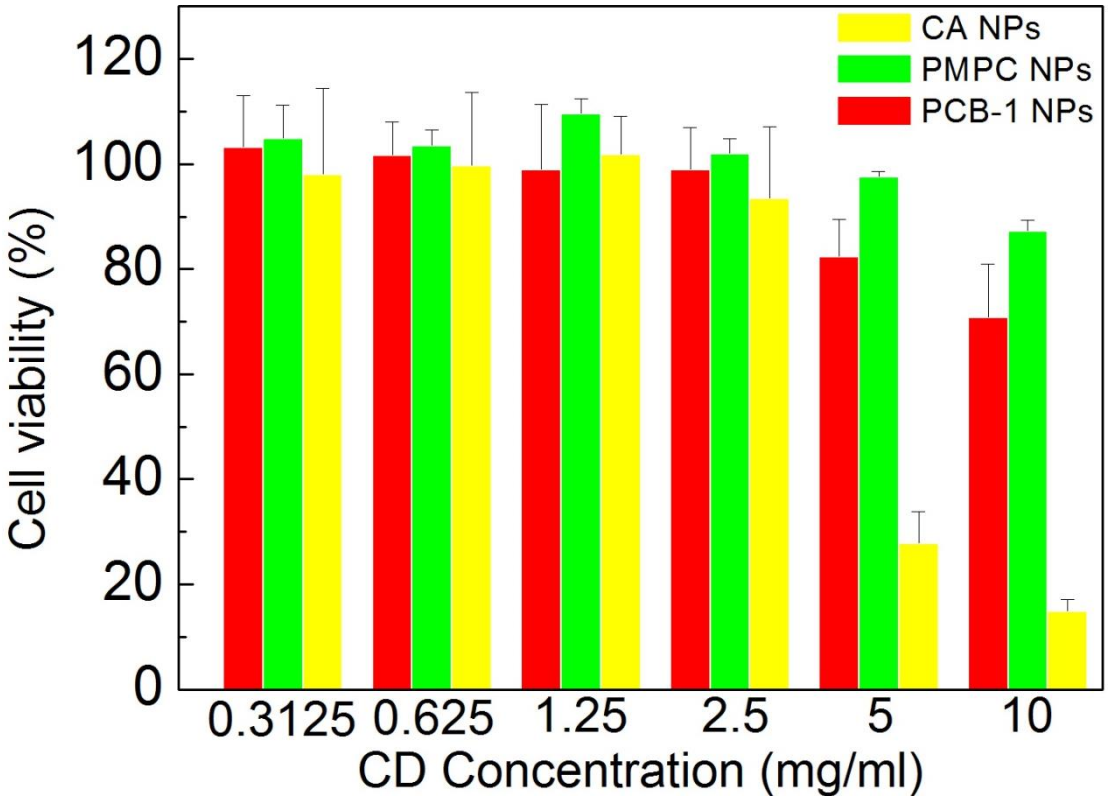

Fig. S6 MTT results of CB-1 , MPC and CA NPs.

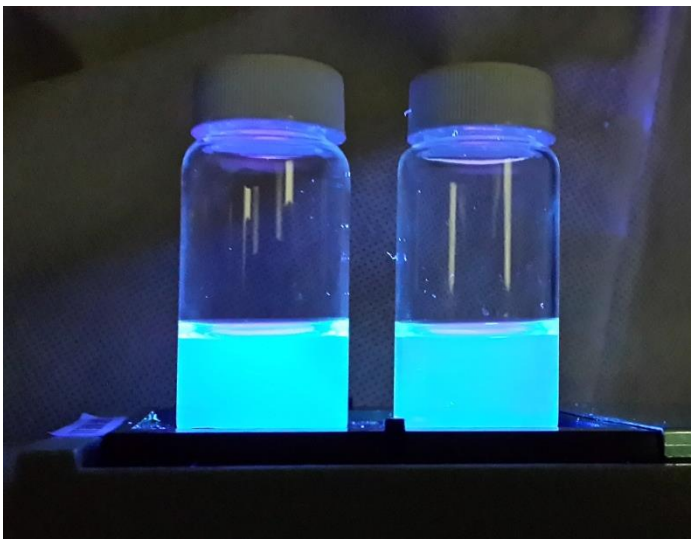

Fig. S7 Fluorescent property of zwitterionic NPs under UV (365 nm) excitation (Lift: PMPC NPs, Right: PCB-1 NPs)

Reference:

1. Cao, Z. Q.; Yu, Q. M.; Xue, H.; Cheng, G.; Jiang, S. Y. *Angew. Chem. Int. Edit.*, 2010, **49**, 3771.