

# Supporting information

## **Highly water-stable, luminescent, and monodisperse polymer-coated CsPbBr<sub>3</sub> nanocrystals for imaging in living cells with better sensitivity**

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### **Experimental section:**

**Materials:** Cesium bromide (CsBr, 99.999% trace metals basis), lead (II) bromide (PbBr<sub>2</sub>, 99.999% trace metals basis), N, N-dimethylformamide (DMF, anhydrous, 99.8%), (3-aminopropyl)trimethoxysilane (APTMS, 97%), oleic acid (OAc, 90%), oleylamine (OAm, 70%), poly(vinyl pyrrolidone) (PVP, avg. Mn 6000, PDI ≤1.2), N-isopropylacrylamide (NIPAM, 97%), acetonitrile (ACN, 99.95%), toluene (anhydrous, 99.8%), ethanol (anhydrous, 99.8%) were purchased from Sigma Aldrich company. n-Octylammonium bromide (OABr, 99%) was purchased from Greatcell Solar Materials company. All chemicals were used without further purification.

**Synthesis of CsPbBr<sub>3</sub> NCs:** CsPbBr<sub>3</sub> NCs were synthesized via a modified LARP method.<sup>1-3</sup> The precursor solutions were prepared by dissolving 0.02 mmol CsBr in 250 μL DMF and 0.02 mmol (7.34 mg) PbBr<sub>2</sub> in 250 μL DMF under vigorous stirring conditions. Next, 250 μL of CsBr precursor was injected into the PbBr<sub>2</sub> precursor solution followed by 50 μL of OAc and 25 μL of OAm to form the final precursor. 250 μL of the final precursor was injected dropwise into a round bottom flask containing 5 mL toluene under vigorous stirring conditions. The reaction was continued for 5 mins. The resulting CsPbBr<sub>3</sub> NCs solution was transferred to a centrifuge tube and 2 mL of ACN was added. The mixture was centrifuged at 6000 rpm of speed for 10 min and the precipitate was dispersed in 1 mL toluene for further experimental characterizations. The sample is renamed **CPB-1** NCs for better understanding.

**Synthesis of CsPbBr<sub>3</sub>@(OA)<sub>2</sub>PbBr<sub>4</sub> NCs:** CsPbBr<sub>3</sub>@(OA)<sub>2</sub>PbBr<sub>4</sub> NCs were synthesized via the modified LARP method.<sup>1-3</sup> First, three separate precursor solutions were prepared by dissolving 0.02 mmol CsBr in 250 μL DMF, 0.02 mmol OABr in 250 μL DMF, and 0.02 mmol (7.34 mg) PbBr<sub>2</sub> in 250 μL DMF under vigorous stirring condition. Next, 200 μL of CsBr precursor and 50 μL of OABr precursor (CsBr:OABr = 8:2) was injected to PbBr<sub>2</sub> precursor solution and mixed properly. Finally, 50 μL of OAc and 25 μL of OAm were added into the mixture solution to form the final precursor. 250 μL of final precursor was injected dropwise into a round bottom flask containing 5 mL toluene under vigorous stirring. The reaction was continued for 5 mins. The resulting NCs solution was transferred to a centrifuge tube and then 2 mL of ACN was added. The mixture was then centrifuged at 6000 rpm of speed for 10 min and the precipitate was dispersed in 1 mL toluene for further experimental characterizations. The sample was renamed **CPB-2** NCs for better understanding.

**Synthesis of PVP-coated CsPbBr<sub>3</sub>@(OA)<sub>2</sub>PbBr<sub>4</sub> NCs:** PVP-coated CsPbBr<sub>3</sub> NCs were synthesized via the modified LARP method.<sup>1-3</sup> First, three separate precursor solutions were prepared by dissolving 0.02 mmol CsBr in 250 μL DMF, 0.02 mmol OABr in 250 μL DMF, and 0.02 mmol (7.34 mg) PbBr<sub>2</sub> in 250 μL DMF under vigorous stirring condition. Next, 200 μL of CsBr precursor and 50 μL of OABr precursor (CsBr:OABr = 8:2) was injected to PbBr<sub>2</sub> precursor solution and mixed properly. Finally, 50 μL of OAc and 25 μL of OAm were added into the mixture solution to form the final precursor. 250 μL of final precursor was injected dropwise into a round bottom flask containing 5 mL toluene and 4 mg PVP under vigorous stirring. The reaction was continued for 30 mins to ensure proper coating of polymer on the NC surface. The resulting NCs solution was transferred to a centrifuge tube and then 2 mL of ACN was added. The mixture was then centrifuged at 6000 rpm of speed for 10 min and the precipitate was dispersed in 1 mL toluene for further experimental characterizations. The sample was renamed **CPB-3** NCs for better understanding.

**Synthesis of PVP and NIPAM coated CsPbBr<sub>3</sub>@(OA)<sub>2</sub>PbBr<sub>4</sub> NCs:** PVP coated CsPbBr<sub>3</sub> NCs were synthesized via the modified LARP method.<sup>1-3</sup> First, three separate precursor solutions were prepared by dissolving 0.02 mmol CsBr in 250 μL DMF, 0.02 mmol OABr in 250 μL DMF, and 0.02 mmol (7.34 mg) PbBr<sub>2</sub> in 250 μL DMF under vigorous stirring condition. Next, 200 μL of CsBr precursor and 50 μL of OABr precursor (CsBr:OABr = 8:2) was injected to PbBr<sub>2</sub> precursor solution and mixed properly. Finally, 50 μL of OAc and 25 μL of OAm were added into the mixture solution to form the final precursor. 250 μL of the final precursor was injected dropwise into a round bottom flask containing 5 mL toluene and 4 mg PVP and 15 mg

NIPAM under vigorous stirring. The reaction was continued for 30 mins to ensure proper coating of polymer on the NC surface. The resulting NCs solution was transferred to a centrifuge tube and then 2 mL of ACN was added. The mixture was then centrifuged at 6000 rpm of speed for 10 min and the precipitate was dispersed in 1 mL toluene for further experimental characterizations. The sample was renamed **CPB-4** NCs for better understanding.

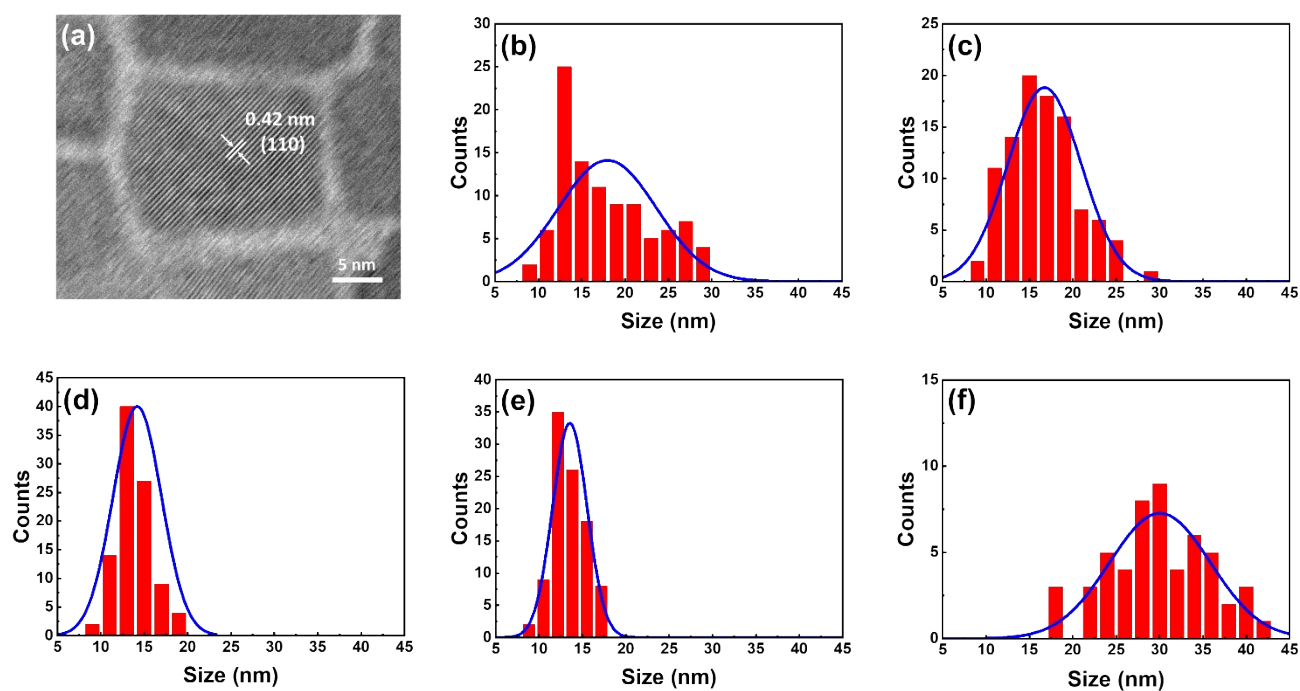
**Synthesis of  $\text{SiO}_2$ -coated  $\text{CsPbBr}_3$  NCs:** Silica-coated  $\text{CsPbBr}_3$  NCs were synthesized via the modified LARP method.<sup>1-3</sup> First, three separate precursor solutions were prepared by dissolving 0.02 mmol (4.2 mg)  $\text{CsBr}$  in 250  $\mu\text{L}$  of DMF, 0.02 mmol (4.2 mg)  $\text{OABr}$  in 250  $\mu\text{L}$  of DMF, and 0.02 mmol (7.34 mg)  $\text{PbBr}_2$  in 250  $\mu\text{L}$  of DMF were mixed properly via stirring to complete dissolution. Next 200  $\mu\text{L}$  of  $\text{CsBr}$  precursor and 50  $\mu\text{L}$  of  $\text{OABr}$  precursor ( $\text{CsBr}:\text{OABr} = 8:2$ ) was injected to  $\text{PbBr}_2$  precursor solution and was mixed properly. Finally, 10  $\mu\text{L}$  of APTMS was injected to the  $\text{PbBr}_2$  precursor to form the final precursor. 250  $\mu\text{L}$  of final precursor was quickly injected dropwise in a round bottom flask containing 5 mL of toluene under vigorous stirring condition. The stirring was continued for 4 hours to complete the hydrolysis process of APTMS. The NCs solution was then centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the precipitate was dispersed in 1 mL of ethanol for further characterization.

**Characterization methods:** UV-visible absorption spectra of NCs were recorded with a Shimadzu UV-2700 Spectrophotometer. Steady-state photoluminescence (PL) spectra were collected with Ocean Insight MAYA 2000 Pro high-sensitivity spectrometer using a 370 nm UV excitation source. X-Ray diffraction (XRD) analysis was carried out with the Bruker D8 diffractometer using  $\text{Cu-K}\alpha$  ( $\lambda=1.54 \text{ \AA}$ ) as incident radiation. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) imaging were recorded by Jeol-JEM-2010 microscope operated at 200 kV to determine the shape and size of the NCs. The Fourier Transform Infrared (FTIR) analysis was carried out using JASCO FT/IR-6600 Infrared Spectrometer to confirm the silica and polymers encapsulation around the NCs. Nuclear magnetic resonance spectra were recorded using an AVANCE III HD 400MHz NanoBay NMR spectrometer. Fluorescence microscopy of NCs thin films was performed using a Nikon Eclipse Ci (Y-TV55) fluorescence microscope. Dynamic light scattering (DLS) analysis was done using a Malvern Zetasizer Nanoseries ZEN3600 DLS instrument. The contact angle of NCs in thin-film form was recorded using a Kyowa DME-211 contact angle meter.

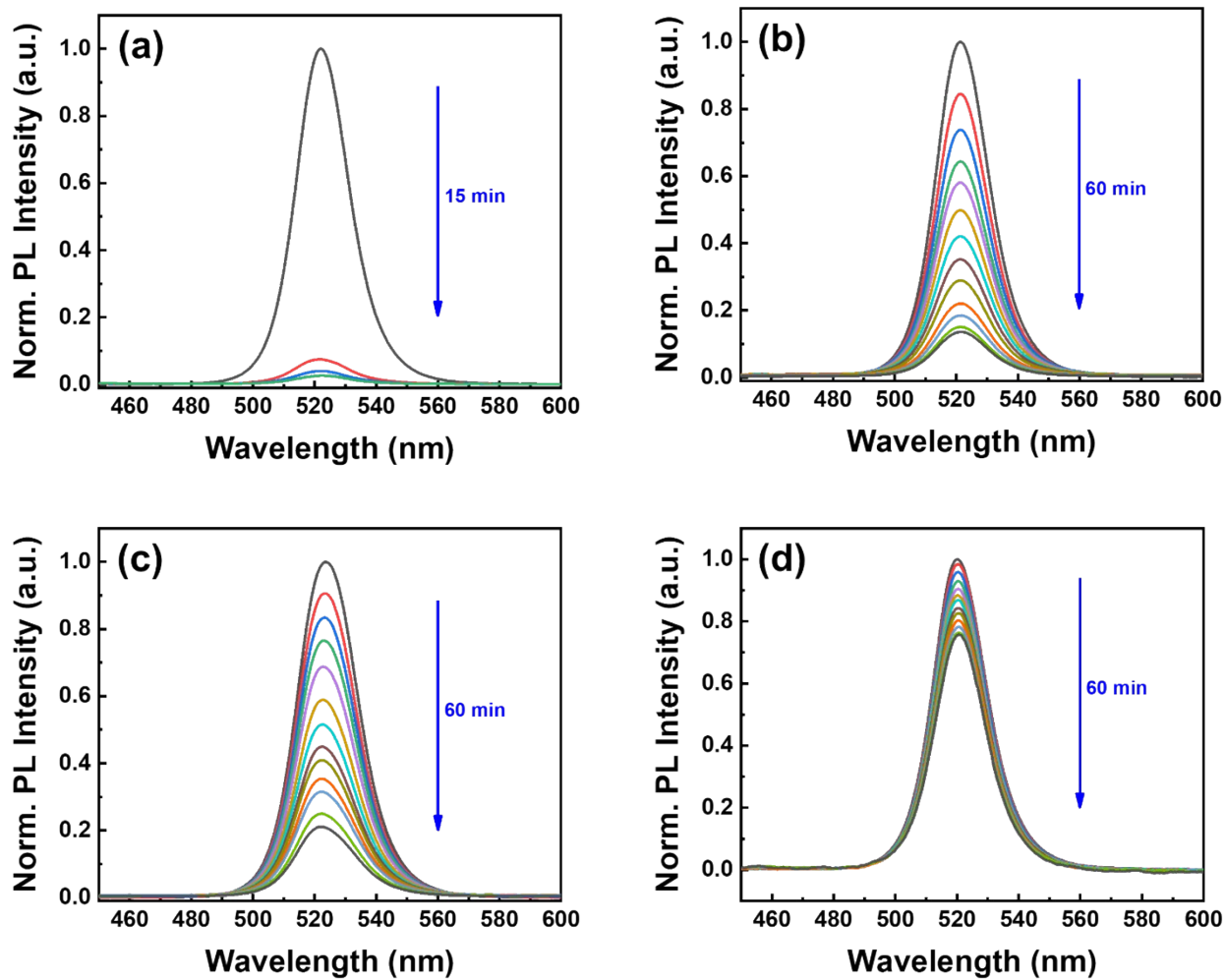
**Mammalian cell culture and live cell imaging:** For bioimaging of CPB-4 NCs, we used Chinese Hamster Ovary (CHO-K1) mammalian cell line. CHO-K1 cells were cultured in Ham's F-12 complete media (HIMEDIA) containing 10% Fetal Bovine Serum (FBS, HIMEDIA). Cells were seeded in 25 mm coverslips and CPB-4 NCs were added to the culture media at a dilution of 1:250 for 1 hr and kept in the incubator at 37° C. After incubation cells were labeled with either LysoTracker Red (Invitrogen, 0.5 µM) or MitoTracker Red CMXRos (Invitrogen, 0.5 µM) for 15 min. Subsequently, the cells were washed with warm F-12 media and taken for live cell imaging. For bioimaging of silica-coated NCs, the cells were treated with the NCs at a dilution of 1:100 in a complete medium. Post incubation cells were stained for mitochondria (Nonyl Acridine Orange; 500 nM) and lysosoma (Lysotracker Red; 500 nM) for 15 min. Live cell imaging was performed using a confocal microscope (Olympus FV3000) with a 63X objective. For long term bio-imaging, CHO-K1 cells were seeded on 35 mm glass bottom dish (IBIDI) and incubated with CPB-4 NCs at 1:250 dilution. Live cell confocal images were taken after 24, 48 and 72 hours of incubation of the NCs. For imaging of NCs, the excitation was done using a 405 nm laser. Images were processed and analyzed using ImageJ software.

**Cell viability assay:** The cell viability after prolonged incubation with the CPB-4 NCs was determined using the MTT assay. Equal number of CHO-K1 cells were seeded on 24-well plate (Eppendorf) and grown till 85-90% confluency. Cells were then incubated with CPB-4 NCs at 1:250 dilution for 24, 48 and 72 hours. Following the respective incubation periods, bright field images were acquired using the Olympus CKX 41 microscope. The media was then removed and cells were incubated with 0.1 mg/ml of MTT solution (HiMedia) in 1X PBS for a period of 5 hours. MTT crystals were visible then and the solution was carefully taken out without disturbing the crystals. DMSO was used to dissolve the crystals and absorbance was taken at 570 nm using Varioskan Multiplate Reader (ThermoFisher). Graphs were plotted using GraphPad Prism 8. In all the cases, the values were normalised with the control of 24 hours.

## Results and discussion:



**Fig. S1** (a) HRTEM image of CPB-4 NCs. (b) Particle size distribution of CPB-1, (c) CPB-2, (d) CPB-3, (e) CPB-4, and (f) silica-coated CsPbBr<sub>3</sub> NCs.



**Fig. S2** Decrease in PL intensities of (a) CPB-1, (b) CPB-3, (c) CPB-3, and (d) CPB-4 NCs dispersed in DI water at continuous time interval of 5 min.

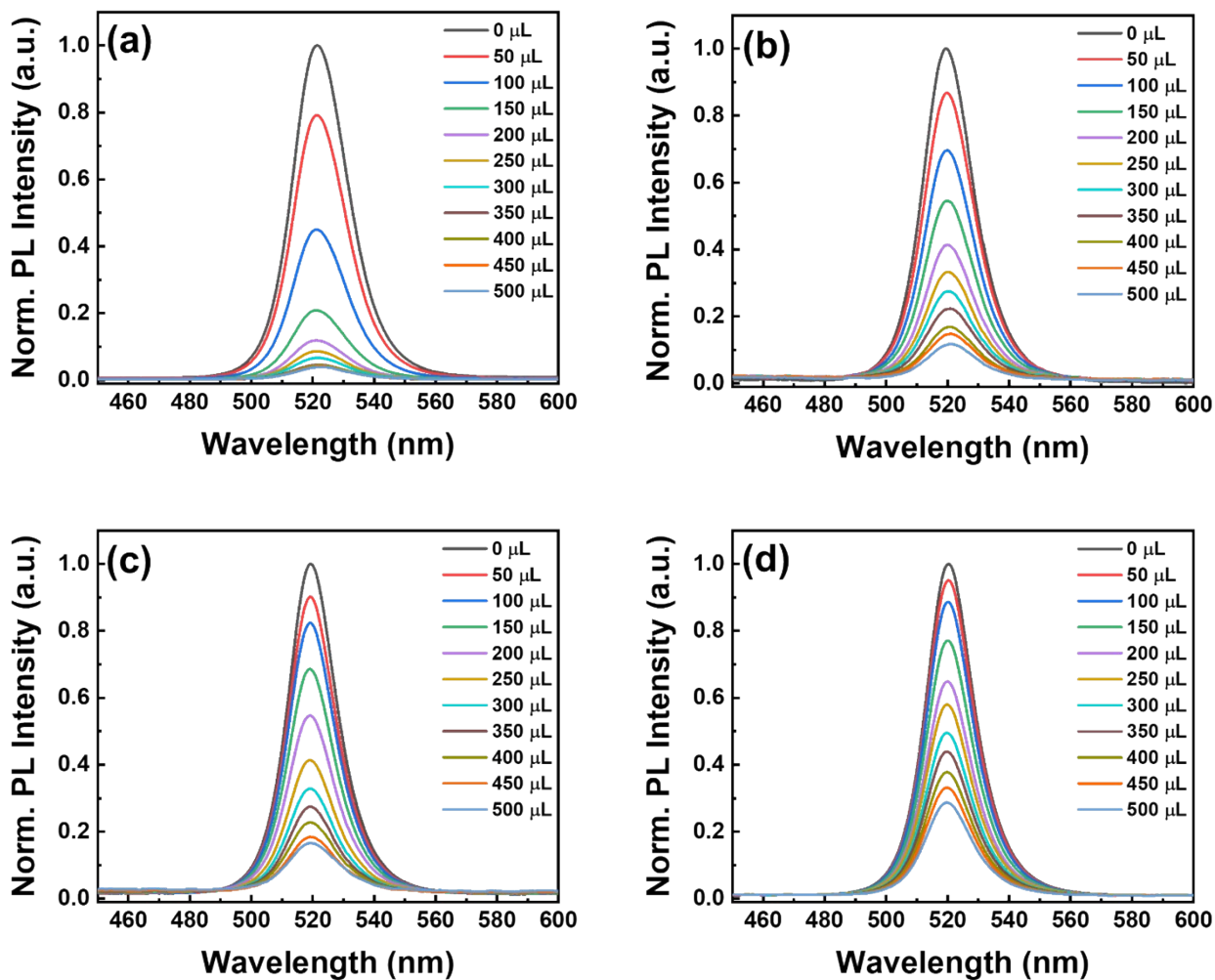
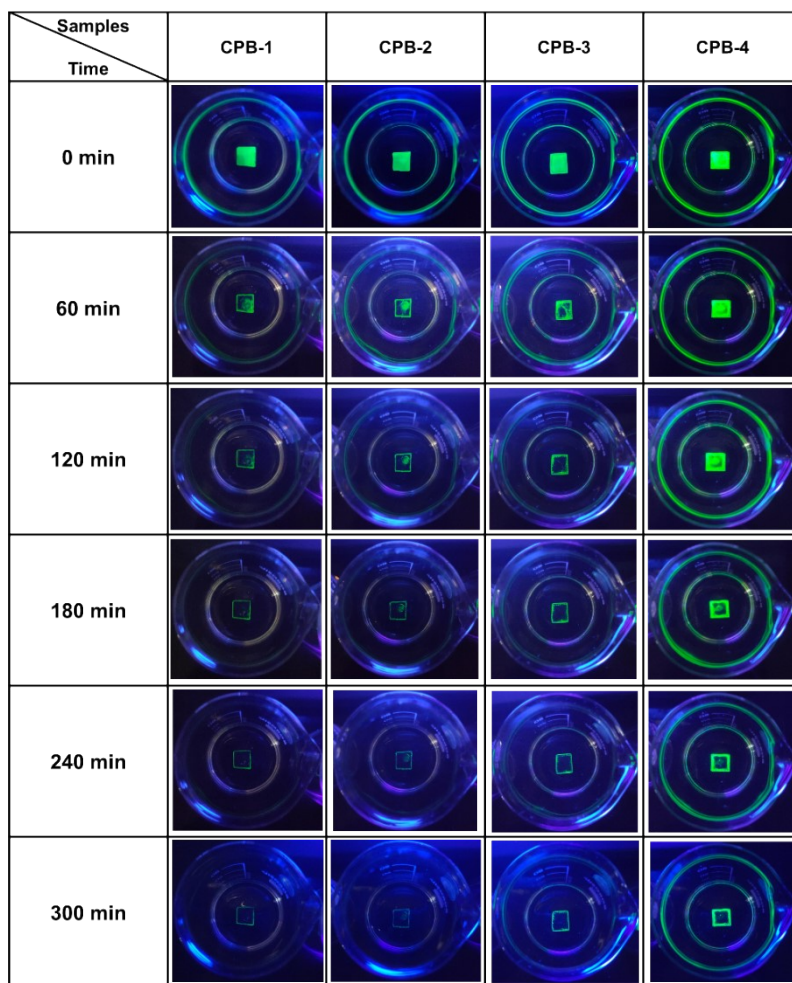
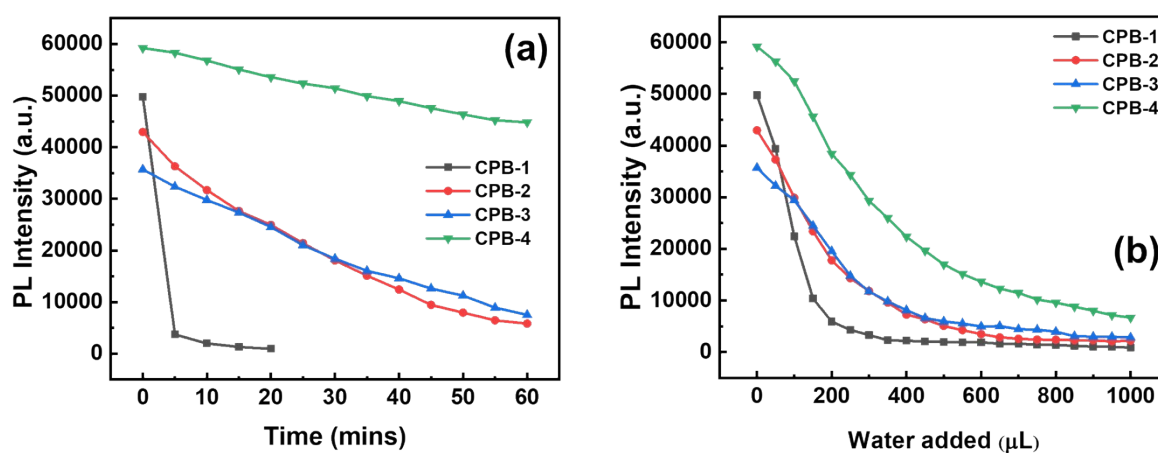


Fig. S3 PL spectra of (a) CPB-1, (b) CPB-3, (c) CPB-3, and (d) CPB-4 NCs solution after different amounts of DI water were added to the corresponding NCs solution, as shown in legends.



**Fig. S4** Photographic images of different NCs films dipped in DI water at a continuous time interval of 60 min under a UV lamp.



**Fig. S5:** The decay curves represent the decrease in PL intensities of CPB-1, CPB-2, CPB-3, and CPB-4 NCs after the addition of DI water as a function of (a) time and (b) amount of water added, as shown in legends.



## Notes and references

1. M. R. Kar, U. Patel and S. Bhaumik, Highly stable and water dispersible polymer-coated CsPbBr<sub>3</sub> nanocrystals for Cu-ion detection in water, *Materials Advances*, 2022, DOI: 10.1039/D2MA00719C.
2. M. R. Kar, R. Chakraborty, U. Patel, R. Chakraborty, S. Ray, T. K. Acharya, C. Goswami and S. Bhaumik, Impact of Zn-doping on the composition, stability, luminescence properties of silica coated all-inorganic cesium lead bromide nanocrystals and their biocompatibility, *Materials Today Chemistry*, 2022, **23**, 100753.
3. S. Bhaumik, S. A. Veldhuis, Y. F. Ng, M. Li, S. K. Muduli, T. C. Sum, B. Damodaran, S. Mhaisalkar and N. Mathews, Highly stable, luminescent core–shell type methylammonium–octylammonium lead bromide layered perovskite nanoparticles, *Chemical Communications*, 2016, **52**, 7118-7121.