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Achieving Solidification and Redispersion of Semiconducting Polymer Dots by Layered Double Hydroxide Incorporation

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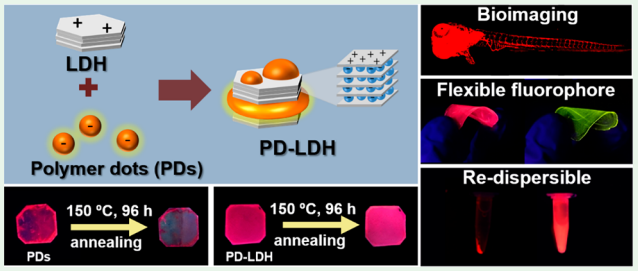
Supporting Information

ABSTRACT: Solidification and aqueous redispersion of fluorophores are characteristically challenging because of aggregation quenching and random shifts of emission. Carbon-based polymer dots (PDs) have recently shown photoluminescent (PL) performances superior to those of conventional dyes and inorganic quantum dots (QDs) but suffer from significant fluorescence (FL) quenching and irreversible aqueous dispersion after solidification, which limits their applications in practical biomedicine and optical devices. In this work, we have compared three strategies by utilizing layered double hydroxides (LDHs) to effectively solidify PDs as robust and redispersible composites with well-preserved high brightness against wide pH (pH = 4−12) and strong ionic strength conditions, more stable than the pristine PDs. The resulting PD−LDH nanocomposites also show in vitro/in vivo bioimaging ability with low cytotoxicity and improved photostability. In addition, these composites exhibit high thermal stability at 150 °C for at least 96 h. The composites produced by a one-step physical mixing approach (~10 min) of LDHs and PDs (p-PLDH) exhibited PL performances comparable to those of the conventional multistep composites (m-PLDH), which require tremendous effort (at least 7 days) to prepare. Structural studies further revealed that the deshaping behavior of PDs and the interflake sandwich assembly of LDHs contributed to the robust FL properties. Because of the simple synthesis, durable photostability, high aqueous redispersion, and multicolor emissions (ranging from blue to near-IR) of these PD−LDH nanocomposites, we believe that they are promising candidates for advanced biorelated technologies and optoelectronics.



KEYWORDS: *layered double hydroxides, polymer dots, bioimaging, solidification, photoluminescence, sandwich assembly*

1. INTRODUCTION

Photoluminescent (PL) stable materials are highly desired for emission devices, biological imaging, and specific labeling.[1−4] However, their solidification and aqueous redispersion without sacrificing PL properties are difficult to achieve because of aggregation quenching and unpredictable shifts of emission colors. Optical device miniaturization with concentrated brightness relies on the success of fluorophore solidification, while aqueous redispersion capability governs the probing stability and diversity in biological imaging. Great efforts have been made to address these issues for high photostability, long-term storage, and convenient device engineering. For example, solidification of carbon dots (CDs) has been demonstrated by poly(vinyl alcohol) (PVA) to generate soft grating films with bright emission, but the shift of the emission wavelength, relatively low thermal stability (<400 K), and nonredispersibility remain unsolved.[5] Silane-mediated solidification on quantum dots (QDs)[6] and CDs[7] has been widely carried out by surface passivation to realize aggregation-free condensation. Improved emission properties are accomplished, yet high material stability is required because of the harsh reaction conditions and complicated procedures adopted in the synthesis. In fact, reversible solidification and aqueous redispersion are characteristic issues in diverse light-emitting materials [e.g., CDs, QDs, molecular dyes, polymer dots (PDs), etc.] for specific applications.[2,5,8,9]

Recently, semiconducting PDs have attracted significant attention because of their superior brightness, quantum yields (QYs), absorptivity, radiative rates, and photostability compared to conventional small organic dyes and inorganic QDs.[10−16] These carbon-based PDs have been approved to be noncytotoxic and more functionalization-capable than inorganic QDs, thus emerging as a new class of PL[17−20] and photoacoustic[21−23] probes for biomedical applications.[24,25] Nevertheless, similar to the issues above, solidification of PDs suffers from nonreversible aggregation (huge size increase after redispersion), reduced PL efficiency, and a loss of bioconjugation capability. Thus, PDs might need to be prepared freshly for immediate use because their PL efficiency might not remain unchanged for very long if no appropriate storage conditions (e.g., low temperature and minimal exposure to light) were adopted. This could greatly limit their potential for presynthesizing the ready-to-use PD colloidal products for clinical and industrial usage. Chiu's group has recently

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developed a lyophilization process for the long-term storage of PD bioconjugates, but the method requires the PDs to be constantly stored at −80 °C.[26] Usually once PDs are dried out from aqueous solutions into the solid-state form, it is very difficult to gain redispersed aqueous PD colloids again by simply adding water to the condensed PD powders.

Layered double hydroxides (LDHs) are versatile layered materials with the general formula

$$[M^{II}_{1-x}M^{III}_{x}(OH^{-})_2]^{x+}[(A^{n-})_{x/n}\cdot yH_2O]^{x-}$$

(M = metals and A = intercalated anions). Different PL materials,[27−31] including fluorescent dyes[8], biomolecules,[32,33], QDs,[9,34,35] metal nanoclusters,[36] metal complexes[37], and room temperature phosphorescent materials,[38] have already been incorporated into LDHs and exhibit sustained PL properties. Compared to other protective materials, LDHs with adjustable size, low cytotoxicity, and weak fluorescent interference are advantageous to realizing PL-reserved solidification. However, conventional LDH hybridization commonly involves exfoliation[36,37], which needs complicated procedures and harsh conditions like high basicity, high temperature, and long operation time. Alternative methods, such as coprecipitation[38−40] and hydrothermal,[41] have also been developed. To simplify that into a one-pot reaction, harsh conditions of high basicity and reaction temperatures should be addressed.

Herein, we have developed three various solidification methods to well preserve the fluorescence (FL) of PDs via incorporation with LDHs (PD−LDH). All of the solidified, redispersible PD−LDH nanocomposites exhibited robust photostability (remained almost unchanged for at least 96 h) under various pH conditions (pH = 4−12) and strong ionic-strength environments. In particular, the physical mixing synthesis (p-PLDH) with a facile (as short as ∼10 min) and single-step procedure at room temperatures represents the most efficient solidification process, rather than cryogenic and harsh preparation for days. The LDH nanoflakes manage to quickly sandwich PDs closely associated with their dimensional mismatch and soft nature of PDs, providing practical synthetic designs capable of the large-scale and easy preparation of exfoliation-free, highly bright, and stable PL nanocomposites. We further applied these PD−LDH nanocomposites for in vitro/in vivo bioimaging to demonstrate their biological applications.

2. EXPERIMENTAL SECTION

2.1. Synthesis of LDHs and PDs. For the synthesis of nanosized LDHs with diameters of around 100 nm, an aqueous solution (90 mL) of $Mg(NO_3)_2\cdot 6H_2O$ (2 M) and $Al(NO_3)_3\cdot 9H_2O$ (1 M) was added to 300 mL of a NaOH solution (0.6 M), followed by refluxing at 100 °C for 24 h.[29] The white precipitate was then centrifuged (8000 rpm) and washed with deionized water several times. The products were dried at 60 °C to obtain pristine LDHs. The LDH [Mg]/[Al] ratio of 2.89 was determined using inductively coupled plasma mass spectrometry (ICP-MS), giving the formula $[Mg^{II}_{0.74}Al^{III}_{0.26}(OH)_2]^{0.26+}[(NO_3)^{-}_{0.26}\cdot nH_2O]^{0.26-}$. For the synthesis of PDs, we added 200 μL of a semiconducting polymer solution [1 mg/mL in tetrahydrofuran (THF)] and 20 μL of PS−PEG−COOH (2 mg/mL in THF), where PS = polystyrene and PEG = poly(ethylene glycol), into 5 mL of THF. The solution was mixed well and then quickly injected into 10 mL of water under vigorous sonication. After that, THF was removed at 96 °C under a N_2 atmosphere to obtain a PD solution.[12]

2.2. Synthesis of a Multistep PD−LDH (m-PLDH). In a typical preparation of m-PLDH, the pristine LDH underwent ion exchange, exfoliation, and recombination with negatively charged PDs. Ion exchange was conducted by adding 0.5 g of LDH powder to a 500 mL aqueous solution of 2 M NaCl and 3.3 mM HCl.[34,42−46] The mixture was stirred under N_2 for 3 days, followed by centrifugation, washing, and drying procedures. The resultant (0.3 g) was added into 300 mL of formamide, and the mixture was stirred under N_2 for another 3 days.[47,48] The supernatant after centrifugation at 2000 rpm was collected for another centrifugation at 14000 rpm (5 min). The dried sediment (5 mg) as exfoliated LDH was dispersed into 1 mL of a PD solution (absorbance at 450 nm is 0.44) and then stirred in the dark for 3 h. The above procedures were all operated at room temperature. The mixture was then washed and dried.

2.3. Synthesis of Single-Step PD−LDH (s-PLDH). A mixture of $Mg(NO_3)_2\cdot 6H_2O$ (2 M, 3 mL) and $Al(NO_3)_3\cdot 9H_2O$ (1 M, 3 mL) was added with 4 mL of NaOH (0.6 M) and then 6 mL of a PD solution rapidly in a Teflon autoclave liner.[29] The resultant was then hydrothermally treated at 100 °C for 8 h. The product was washed, dried, and denoted as s-PLDH. For FL measurement, 8.7 mg of the powder was dispersed in 1 mL of water for further dilution.

2.4. Synthesis of Physically Mixed PD−LDH (p-PLDH). The pristine LDH (5 mg) was mixed with 1 mL of PD and stirred until homogeneously suspended for around 3 min. The mixture was washed by water and centrifuged at 14000 rpm for 5 min.

2.5. Synthesis of the PLDH−PVA Film. For the PLDH−PVA film preparation, the addition of 1 mL of a PLDH suspension into 2 mL of a PVA solution (20 wt %) was conducted. Here we used a 20% (w/w) PVA solution because of the modest solubility of PVA (M_w = 89000−98000) in 70 °C hot water and the appropriate mechanical properties as bendable films. After gentle stirring to a homogeneous mixture, the solution was transferred to culture plates and dried under ambient conditions.

2.6. Materials and Characterization. All chemicals were used directly without further purification. $Mg(NO_3)_2\cdot 6H_2O$ and $Al(NO_3)_3\cdot 9H_2O$ were purchased from Acros, and NaOH was purchased from Shimakyu. The synthesis of PDs with different emission wavelengths was recorded in the literature.[12,49] The PDs with yellow and red FL are [poly(fluorene-*alt*-benzothiadiazole)] (PF-BT) and [poly[(9,9-dioctylfluorene)-*co*-2,1,3-benzothiadiazole-*co*-4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole] (PF-BT-DBT), respectively. The material morphology and crystallinity were determined by field-emission scanning electron microscopy (SEM; JEOL-6330), X-ray diffraction (XRD; Bruker D2 Phaser), and transmission electron microscopy (TEM; JEOL JEM-2100). The FL spectra were acquired with a Hitachi F-7000 spectrometer (excitation wavelength = 450 nm). The emission behavior of PDs and the three PLDH composites were studied by adding 1 mL of dispersed PD or PLDH into 4 mL of water or a buffer solution for the FL intensity measurements, thermal stability experiments, and QY measurements. The buffer solutions include 0.01 and 0.1 M phosphate-buffered saline (PBS) with pH values of 4, 7, and 12 adjusted by HCl or NaOH. The thermal stability experiment was conducted by dropping 500 μL of a PD and PLDH solution on 1 × 1 cm^2 commercial PET films. After drying at 60 °C for 24 h, the films were placed in an oven at 150 °C for a certain time. QYs were acquired by direct methods with FL spectra (Hitachi F-7000) coupled with an integrating sphere. In addition, the zeta potentials of the pristine LDHs, bare PDs, and p-PLDH were measured by a Zetasizer Nano system to be +54, −34, and +47 mV, respectively. The reaction yields of m/s/p-PLDH are estimated by comparing the PL intensity of the colloidal PDs for PLDH preparation and the PL of the supernatant after PD solidification. Typically, the yields of all PLDH samples were in the range of 98−99%.

2.7. Bioimaging Procedures. The transgenic zebrafish, Tg-(kdrl:eGFP)la116 expressing eGFP in the endothelial cells, were kept at 28 °C and bred under standard conditions with approval from National Sun Yat-sen University Animal Care Committee. For angiography imaging, 37 nL of PD (125 nM) in a 20 mM HEPES buffer was injected into the sinus venosus of the anaesthetized zebrafish embryos 3 days postfertilization (dpf) with 5% (v/v) tricaine. After 20 min recovery, the injected embryos were immobilized in 1.5% low-melting-point agarose (Sigma) and then imaged immediately by using a fluorescence confocal microscope (Zeiss LSM 700).

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The cervical cancer cell line HeLa was ordered from Food Industry Research and Development Institute (Taiwan). Primary cultured HeLa cells were grown in Dulbecco's modified Eagle medium (catalogue no. 11885, Life Technologies) supplemented with 10% fetal bovine serum and a 1% penicillin−streptomycin solution (5000 units/mL penicillin G and 50 μg/mL streptomycin sulfate in 0.85% NaCl) at 37 °C with a 5% CO_2 humidified atmosphere. The cells were precultured in a T-25 flask and allowed to grow for 3−4 days prior to the experiments until ca. 85% confluence was reached. To suspend cells, the adherent cancer cells were quickly rinsed with media and then incubated in 0.8 mL of a trypsin−ethylenediaminetetraacetic acid (EDTA) solution (0.25 w/v % trypsin and 0.25 g/L EDTA) at 37 °C for 3 min. The cell suspension solution was then centrifuged at 1000 rpm for 5 min to precipitate the cells to the bottom of the tube. After taking out the upper media, the cells were rinsed and resuspended in 5 mL of culture media. Approximately tens of thousands of cells were split onto a glass-bottomed culture dish and allowed to grow for 12 h before PD tagging. For endocytosis, 100 μL of PLDH solutions was added to the flask containing cells and then incubated for 2 h. Prior to FL imaging, the cells were rinsed with a PBS buffer to remove any nonspecifically bound PLDH on the cell surface.

3. RESULTS AND DISCUSSION

3.1. Synthesis of PD−LDH Nanocomposites. Functionalization of carboxyl (−COOH) groups by blending PS−PEG−COOH on the surface of PD is critical to ensuring their stable dispersion in water and capability of further surface modification.[12] In addition, the negative charges on the surface of PD can also be attributed to polymer oxidation in the nanoprecipitation process by which PD was prepared.[50] After proton dissociation of the carboxyl groups in pH = 7 water, the PD with negatively charged surfaces (i.e., zeta potential of −34 mV) can disperse well because of electrostatic repulsion among the nanoparticles. However, a more acidic condition (pH < 5) would substantially inhibit dissociation of the carboxyl groups, and thus PD would tend to aggregate immediately, leading to FL fluctuation or even PL quenching. This issue could be more severe in pristine PD solidification.

Various LDHs comprised of different di- and trivalent metal ions (M^{2+}/M^{3+}) have been reported.[45,46,51,52] Here we chose Mg/Al-LDH as our protecting material for PD because it has exhibited high biocompatibility and no quenching effect toward fluorescent dyes.[29,52−54] Besides, the surface charge of Mg/Al-LDH is positive and can combine well with the negatively charged PDs via the driving force of electrostatic interactions. In terms of PD, we selected PDs with four different emission colors (blue, PFO; yellow, PF-BT; red, PF-BT-DBT; near-infrared, NIR695-doped PF-BT-DBT; as shown in Figure 1a)[49] to examine the performance of PD−LDH hybrids. It is worth mentioning that yellow (PF-BT) and red (PF-BT-DBT) PDs have already been extensively used for biological and sensing applications because of their relatively high stability. Therefore, we took PF-BT and PF-BT-DBT as examples for the following initial assessments.

We compared three solidification strategies for the preparation of PD−LDH nanocomposites, which are shown in Scheme 1. These strategies include (i) multistep, (ii) single-step, and (iii) physical-mixing methods. For the multistep approach, the synthesized LDHs went through conventional ion exchange and exfoliation first and then were assembled with PDs to form PD−LDH nanocomposites (see the Experimental Section for details). The hybrids formed by the use of multistep procedures are denoted as m-PLDH. Although these complicated syntheses have been widely adopted for molecular dye and QD systems,[34] the overall processes are relatively

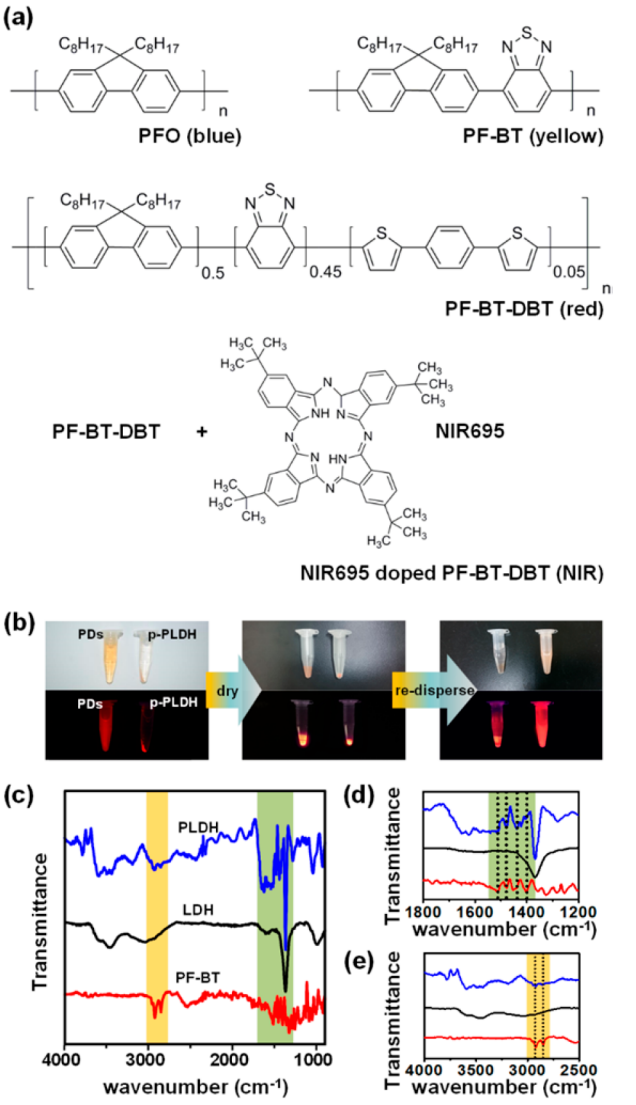

Figure 1. (a) Chemical structures of semiconducting polymers with abbreviations and emission colors. (b) Photographs of PF-BT-DBT PD and PF-BT-DBT p-PLDH after centrifugation at 14000 rpm (left images), subsequently dried to remove water (middle images), and finally redispersed in water (right images). All of the top photographs were acquired under ambient light, and the bottom ones were under a 365 nm UV lamp. (c) FTIR spectra of the PF-BT PD, pristine LDH, and PF-BT p-PLDH. The magnified spectra corresponding to the green and orange regions in part c are shown in parts d and e, respectively.

inefficient and need at least 1 week to obtain the final products. To simplify the tedious synthetic procedures, we conducted the single-step strategy in which PDs were combined directly with metal precursors of LDHs under hydrothermal treatments at 100 °C. During the reaction, the cations of Mg and Al might be attracted adjacent to the negatively charged PDs and then sandwiched PDs directly to form PD−LDH nanocomposites (denoted as s-PLDH).

It has been reported that the physical incorporation of unexfoliated LDH into the polymer matrix can enhance the polymer thermal stability and mechanical properties.[55,56] Therefore, we performed a physical mixing approach by directly blending PDs with unexfoliated LDH to produce PD−LDH hybrids (denoted as p-PLDH). Compared to the

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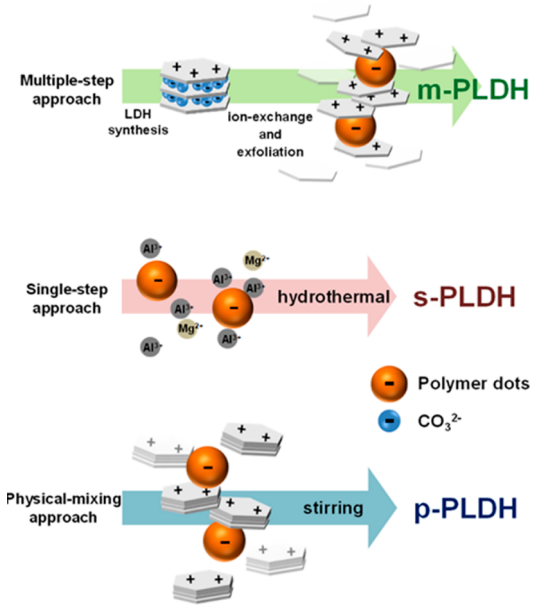
Scheme 1. Schematic Illustration of the Three Approaches for the Synthesis of PD−LDH Nanocomposites



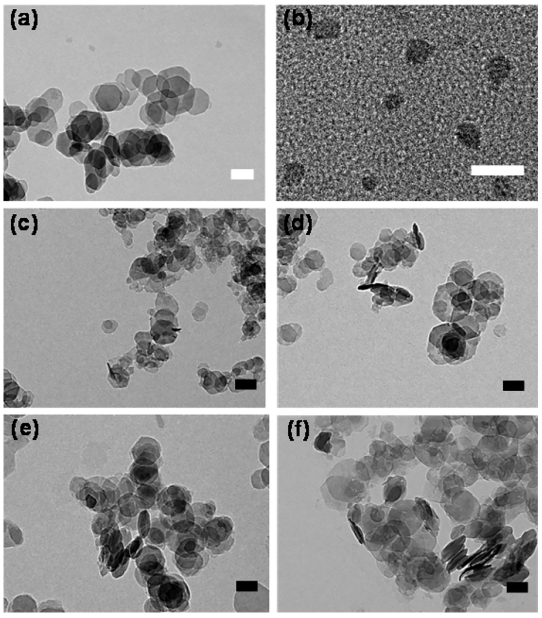


Figure 2. TEM images of (a) pristine LDH, (b) PD, (c) s-PLDH, (d) p-PLDH, (e) m-PLDH, and (f) m-PLDH with double the amount of PD. The scale bar is 50 nm for part b and 100 nm for the rest of the images.

synthesis of s-PLDH and m-PLDH composites, p-PLDH composites are much easier to prepare.

3.2. PD Solidification in PLDH. The solidification and redispersion performances of s-PLDH, m-PLDH, and p-PLDH nanocomposites were evaluated. In the case of PF-BT-DBT as p-PLDH, for example, complete precipitation was observed after centrifugation (left panel in Figure 1b). The supernatant, however, is essentially clear, colorless, and nonfluorescent under UV without any white powder suspension, while the pellet is highly colored and fluorescent under UV light, indicating a high degree of PD encapsulation within LDH. On the contrary, pure PDs were still suspended after centrifugation.

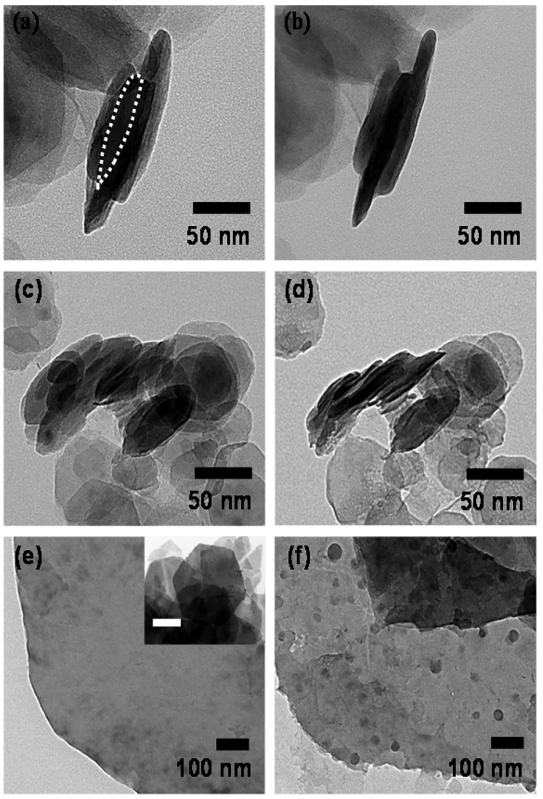


Figure 3. TEM images of m-PLDH composites before (a) and after (b) focused electron-beam irradiation. (c and d) p-PLDH composites before and after focused electron-beam irradiation, respectively. (e) Bulky LDH with a diameter of 2 μm before PD hybridization. The scale bar in the inset corresponds to 1 μm. (f) Bulky LDH after multistep hybridization with PDs.

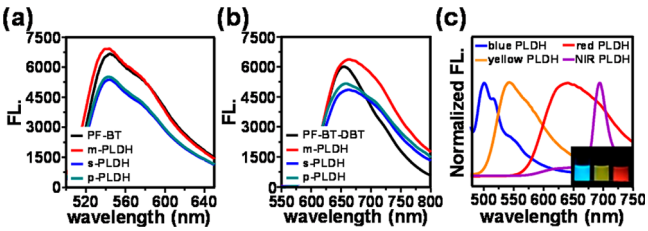


Figure 4. Fluorescent comparison of (a) yellow PDs (PFBT) and (b) red PDs (PFBT-DBT) with the corresponding PLDH composites. (c) m-PLDHs of different emission wavelengths from 500 to 700 nm. The inset picture is the fluorescent photographs of blue, yellow, and red m-PLDH samples corresponding to the spectra in part c.

We subsequently dried out the centrifuged p-PLDH and pure PDs under vacuum (middle panel of Figure 1b) to obtain their solidified products. The PL data show negligible emission change before and after the dry-out step (Figure S1). We then redispersed the solidified p-PLDH and PDs by adding water under vigorous sonication in which p-PLDH composites achieved redispersion. These results have demonstrated that LDH could successfully solidify PDs and at the same time preserve their optical properties for further redispersion.

To verify the successful PD solidification with LDH, we conducted Fourier transform infrared (FTIR) experiments as shown in Figure 1c−e, in which the signature peaks were highlighted with orange and green stripes. The characteristic peaks of PDs at 2850 and 2920 cm^{-1} (the orange region) can be attributed to the symmetric and asymmetric stretching

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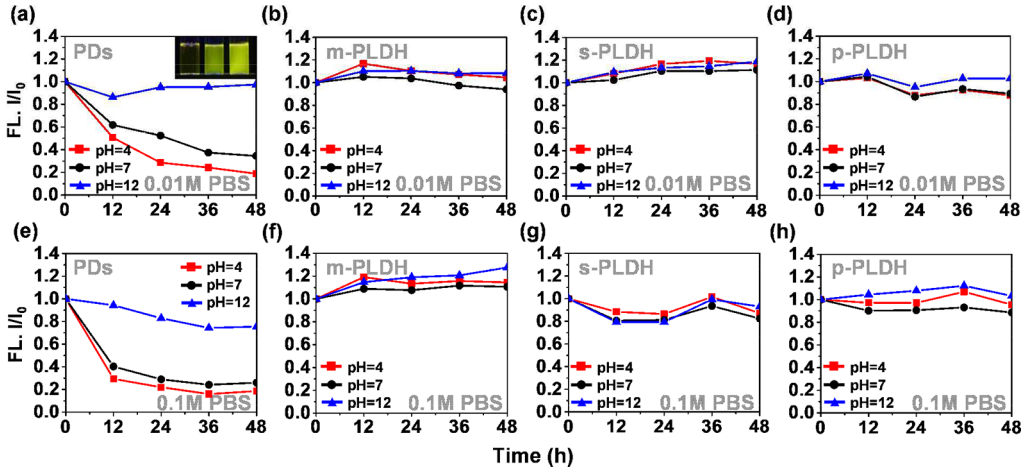

Figure 5. Photostability of the pristine PDs and the PLDH composites under varied pH and ionic strength conditions. The data of parts a–d are acquired in 0.01 M PBS with pH values of 4, 7, and 12; those of parts e–h are recorded by increasing PBS concentration to 0.1 M. The inset in part a shows PF-BT PDs suspended in 0.01 M PBS at pH = 4, 7, and 12 (from left to right, respectively) for 48 h under UV-lamp excitation.

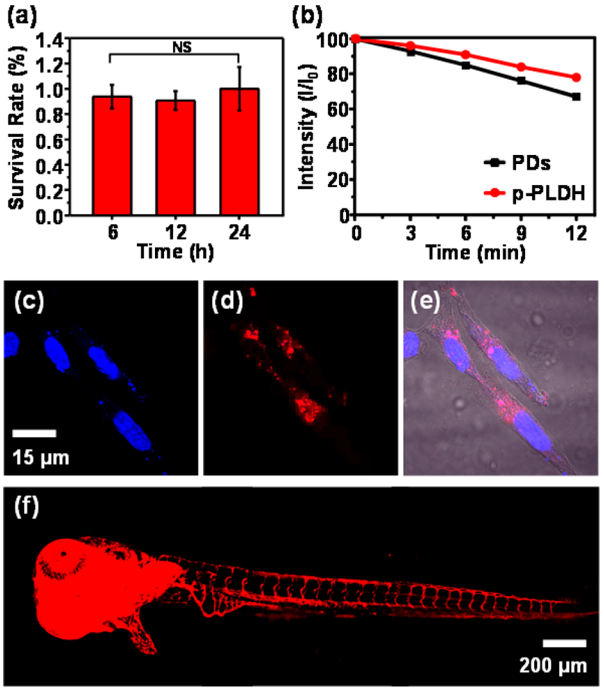

Figure 6. (a) Cytotoxicity tests of PF-BT-DBT p-PLDH with 98% survival rate after 24 h. Values are expressed as the mean ± SD (error bars). The statistical significance was determined by a two-tailed *t* test. (b) Photostability of PDs (Abs = 1) and p-PLDH under continuous irradiation of 408 nm laser. Confocal FL images of HeLa cells labeled with p-PLDH. The blue FL in part c was from nuclear counterstain Hoechst 34580, and the red FL in part d was from p-PLDH. The overlay images are in panels c and d, and the bright-field image is shown in part e. (f) Zebrafish microangiography by injection of p-PLDH with a scale bar of 500 mm.

modes of the C–H bond in alkyl groups, while the signals at 1390–1520 cm^{-1} (the green region) are assigned to the vibrational features of a conjugated polymer chain.[56−59] These signals could be observed in PLDH but were absent in pristine LDH, confirming successful PD solidification with LDH.

3.3. Assembly Studies of PLDH. It is important to understand the structural configurations of the solidification to correlate the preserved PL and high photostability. Figure S3a shows the XRD results of s/p/m-PLDH samples and pristine LDH. All of these composites reveal characteristics similar to those of the intrinsic LDH, suggesting an interlayer spacing identical with that of pristine LDH. The absence of peaks with greater *d* spacing than d_{003} in LDH indicates that no intercalation occurred among the synthesized PLDH samples, including m-PLDH. Thus, LDH might hybridize PDs by sandwiching rather than interlayer intercalation, similar to the layer-by-layer assembly reported by the Wei group.[55] In addition, we examined one of the m-PLDH intermediates, Cl^- ion-exchanged LDH, showing an increased *d* spacing for (003) of 0.19 Å (Figure S3b).[34,45] These results indicate the success of ion exchange, but LDH intercalation with PDs is not favored by following conventional ion exchange, exfoliation, and recombination procedures. Therefore, the organic/inorganic interfacial behavior and material nature of PD–LDH are quite different from that of all inorganic composite systems.[9,34] On the other hand, the synthesized PLDH showing the same XRD patterns to LDH is reasonable, since no synthetic effort of ion-exchange or exfoliation is involved.

To visually monitor the PD–LDH interface, TEM and SEM characterizations were carried out. The pristine Mg–Al LDH, prepared by reflux and hydrothermal methods, shows a characteristic hexagonal shape with the major size distribution of around 80–100 nm, which is appropriate for in vitro cellular labeling and in vivo bioimaging (Figures 2a and S4). As shown in Figure 2b, bare PDs appeared to be of spherical particle shape with diameters of 20–30 nm, in the same order of magnitude as LDH. All three PLDH nanocomposites showed particle-size distributions (105 ± 29 nm for m-PLDH; 92 ± 19 nm for s-PLDH; 95 ± 20 nm for p-PLDH) and morphologies similar to those of the pristine LDH (Figure 2c–e). However, the significant overlapping among PLDH flakes makes the clear identification of PDs very difficult. Therefore, the locations of the PDs and their interfaces with LDH were indistinguishable. The SEM images could not provide the indication of PDs adsorbed on the LDH surfaces (Figure S5).

To identify the locations of the PDs and the hybridization interfaces, we further doubled the solidification quantity of PDs during the preparation of m-PLDH (Figure 2f), but the PDs were still not distinguishable from the LDH flakes. Alternatively, TEM characterization particularly focusing on the

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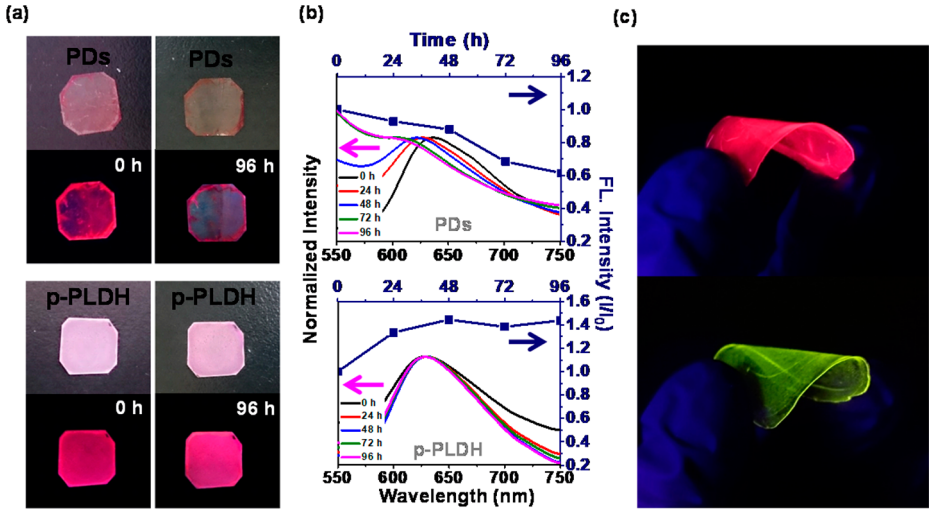

Figure 7. Thermal endurance tests of the bare PD and p-PLDH films. (a) Photograph comparison of PD and PLDH films on PET films after baking at 150 °C for 96 h. The lower images were acquired under 365 nm UV illumination. (b) Normalized FL spectra and the corresponding intensity plots of PD (the upper one) and p-PLDH (the lower one). (c) Flexible films of red and yellow p-PLDH in PVA films.

vertically standing m-PLDH flakes was carried out. The appearance of vertical m-PLDH (Figure 3a) did not seem to encapsulate small spherical PDs between two LDH flakes. Interestingly, we found that many stacks of oval-like flakes (labeled by the white dashed line in Figure 3a) were exhibited. Besides, shrinking behavior with an appreciable decrease of the volume after irradiation of the focused electron beam could be observed (Figure 3b). A similar phenomenon was also observed in p-PLDH, as shown in Figure 3c,d. Because of the robust property of LDH so that it would not undergo deformation under electron-beam irradiation, this shrinkage among the LDH flakes is more likely to be ascribed to PD degradation. This phenomenon suggests that PDs should be sandwiched by and/or adsorbed on the LDH flakes. Besides, the nonspherical morphologies of PLDH (e.g., oval-like assemblies) suggest a deshaping mechanism of the PDs after LDH sandwiching. Unlike rigid inorganic QDs, the carbon-based PDs are essentially soft enough to tolerate high degrees of morphological deshaping.

To confirm the deshaping behavior of hybridized PDs, we prepared wider LDH flakes (creating a drastic size difference from the PD) with a diameter of ~2 μm[60] in order to provide sufficient surface areas for PD solidification with one individual LDH flake (Figure 3e). From TEM characterization, we could clearly observe the location of PDs with enlarged size distributions ranging from 40 to 70 nm (Figure 3f), showing experimental support of the morphological deshaping. Furthermore, the deshaping effect of PDs after hybridization may explain the emission broadening of PLDH (e.g., Figure 2). It has also been reported that imperfect intercalation of the soft biopolymer into LDH could occur after hybridization and further solidification. On the basis of the above experimental results, it is suggested that PDs are sandwiched by LDH flakes after solidification. Because of the comparable sizes between individual LDHs and deshaped PDs, the sandwich interaction (a single LDH only carrying 1−2 PDs) isolates individual PDs from bulky aggregation to obtain a robust fluorophore against harsh pH and strong ionic strength conditions even after solidification.

Although the full structural understanding of PLDH composites is still unclear at this stage, the experimental data strongly support that most PDs should be sandwiched among LDH flakes, regardless of which synthetic method in this study was employed. The PD, therefore, has a small possibility of being adsorbed onto the exposed surfaces of LDH, which affects the FL intensity of PD after incorporation. A similar sandwich incorporation of QD with unexfoliated LDH for light-emitting applications has been reported.[55] The unfavored PD intercalation in LDH galleries is presumably due to the drastic dimensional gap between the thickness of an exfoliated single LDH nanosheet (less than 1 nm) and the diameters of the PDs (20−30 m). According to Gu et al.,[61] the structure assembly of the relatively small LDH nanoflakes (tens of nanometers) and large volume cores (e.g., submicron scales of Fe_3O_4)[62] can lead to core/shell-like structures and varied orientations of the small objects (LDH nanoflakes) rather than LDH intercalation. The dimensional mismatches between PDs and the LDH composites are comparable to those of that model. Despite the absence of PD/LDH intercalation, their hierarchical assembly can be a novel pioneer of superstructured fluorophores. After all, among these PLDH nanocomposites, p-PLDH appeared to have the simplest and most efficient synthesis for achieving PD solidification and redispersion.

3.4. FL Study. Figure 4 shows the FL spectra of PF-BT and PF-BT-DBT PDs and their corresponding PLDH nanocomposites prepared by three different solidification approaches. All of the PF-BT and PF-BT-DBT m/s/p-PLDH nanocomposites showed characteristic emission peaks identical with those of pristine PDs with slight peak broadening (Figure 4a,b), suggesting possible minor morphology changes of PDs after solidification.[63] We found that m-PLDH showed the highest FL intensity compared to s-PLDH and p-PLDH. In the synthesis of s-PLDH, the highly basic preparation involving a strong base of NaOH under pressurized hydrothermal treatments at 100 °C was attributed to the reduced emission of PDs. For p-PLDH hybrids, their emission intensity was slightly higher than that of s-PLDH yet lower than that of m-PLDH. p-PLDH represents the most facile and time-effective approach to obtaining emissive PLDHs whose microstructural assembly and FL are comparable to other PLDHs synthesized through other means in this study; hence, we selected physical

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mixing as the primary synthetic method to exhibit other properties in this work.

To evaluate the FL quenching of PDs after solidification, we measured the QYs of solidified/redispersed PDs in water after the drying-out treatment, as presented in Figure 1b. The results show a quenched QY of 11%, while the redispersed m/s/p-PLDHs give a QY range (see Table S1) close to that of pristine PDs (~30%).[12] The large difference in the QYs between unprotected PDs attempting redispersion and PLDHs means that quenching due to the aggregation of PDs is minimized in the PLDHs. Furthermore, the emission of all of the PLDH composites remains stable for aging (at least 48 h; see also Figure 5) and repeated cycles of solidification/redispersion treatments. These data clearly verify that LDH incorporation is an effective strategy to well preserve the FL of PDs as bright fluorophores against solidification and redispersion without the need for cryogenic conditions in aqueous solutions, which is practically valuable for the manufacturing process of various optical devices. We further measured the FL lifetime by a time-correlated single-photon-counting module system. As shown in Figure S8, we found that the FL lifetime of bare PF-BT-DBT PDs was 4.33 ns and slightly decreased to 3.63 ns after LDH hybridization. This is probably due to some metal ions in LDH that might increase the nonradiative decay rate constant of the PDs. In addition, we further show the incorporation of different types of PDs with diverse fluorescent colors (blue, yellow, red, and near-IR in LDH can also be synthesized; Figure 4c), suggesting that the LDH solidification method used in this study is universal for polymer-based nanoparticles.

3.5. Fluorescent Stability. Because many biological systems are not completely neutral and may contain varied concentrations of different ions, the photostability of all of the solidified PLDHs was examined against harsh aqueous conditions with pH values of 4, 7, and 12. The data confirmed that the as-prepared LDHs can be stable under pH = 4 at least for 1 month (see Figure S2). The effects of the pH on PDs and PLDHs were systematically investigated by FL measurement based on the intensity ratios I/I_0. As shown in Figure 5a, in the case of 0.01 M PBS, the FL intensity of PDs decreases rapidly at pH = 4 in which less than 50% and 20% of PL remained after 12 and 48 h of exposure, respectively. This quenching behavior is ascribed to nanoparticle aggregation after COOH protonation on the PD surfaces. Such quenching can also be easily observed through the naked eye under UV excitation (Figure 5a, inset). The FL intensity of PDs in a neutral buffer solution was gradually quenched because of the large ionic strength. In the pH = 12 conditions, PDs showed a decreased FL immediately, followed by a recovery or even a slightly increased emission intensity (vide infra Figure 5a). This phenomenon might be associated with the increase of the ionic strength and dissociation of the COOH groups. In contrast, the FL intensities of m/s/p-PLDH were very stable under different pH conditions (Figure 5b–d). This indicates that the presence of LDH can greatly preserve the optical stability of PDs because LDH may isolate the hybridized PDs from protonation and sever aggregation. At this stage, no performance difference can be observed among the products by the three solidification methods.

It is known that the FL stability of PDs depends on the quantity of surface charges, particularly under conditions of high ionic strength.[16,64] The photostability of bare PDs and PLDHs was evaluated and compared with increasing ionic strengths. As shown in Figure 5e–h, we examined the optical stability of PDs and PLDHs in 0.1 M PBS (10 times more concentrated than the widely adopted 0.01 M environment).[65] The emission intensities of bare PDs were severely affected in 0.1 M PBS and even more seriously in acidic conditions. Despite the higher ionic strength conditions, all of the PLDH composites still displayed high photostability at different pH conditions. According to Jin et al.,[64] the successful improvement of the PD colloidal stability through surface polyelectrolyte coating has been achieved under 1× PBS. Our results show that even greater photostability of PDs against 10× PBS conditions is now feasible via PD–LDH solidification. Because of the high photostability, good FL QY, and facile synthetic procedures of p-PLDH, we selected p-PLDH for further bioimaging applications and thermal endurance assessment.

3.6. Bioimaging. The toxicity of bare PDs was previously tested by several different groups, suggesting minimal cytotoxicity of PDs.[16,49,66] We here examined the toxicity of PF-BT-DBT p-PLDH by using MTT assay and found out that PF-BT-DBT p-PLDH has a minimal cytotoxic effect on cells while maintaining photostability under a 408 nm laser, as seen in Figure 6a,b. This is probably due to the protection of LDH against photooxidation, thus allowing a longer operation time of fluorescent probing than that of pristine PDs in vivo. We also labeled the cells with PF-BT-DBT p-PLDH via endocytosis. Parts c–e of Figure 6 show the confocal microscopy images of HeLa cells after the endocytosis of p-PLDH, clearly revealing that p-PLDH nanocomposites could be labeled on cells for advanced biological applications.

For in vivo imaging, we first added PD solutions with different concentrations into zebrafish embryos. We then injected p-PLDH into the sinus venosus of the zebrafish larva, and the red FL from p-PLDH could be clearly observed immediately in the lumen of the vessels (Figure 6f). These results, together with cellular labeling, demonstrated that p-PLDH exhibits excellent biocompatibility and optical stability, which are ideal for biological imaging.

3.7. Thermal Endurance. Solid fluorophores are important candidates in various nonlinear optical and optoelectronic devices. For these applications, the thermo- and photostability of solidified fluorophores are critical concerns. We carried out thermal endurance tests with p-PLDH and the bare PD as the comparison reference. Figure 7a shows the dropcasting of PD and p-PLDH layers onto a transparent PET substrate (1 × 1 cm^2), showing that the solid-state p-PLDH formed a uniform film coating much more easily than PD. The uneven distribution of PD could be presumably due to PD aggregation under a solvent-free environment. The emission of the PD layers faded away after 96 h of baking at 150 °C, and the remaining FL could be seen only at the edges (Figure 7a). In addition, the maximum emission wavelength of PD films exhibits a blue shift with increased heating time (Figure 7b), accompanied by an intensity decrease (at 640 nm) to 60% after 96 h of heating. The blue shift of the PD films can be attributed to degradation of the conjugated polymer chain inside PD[63,67]. On the other hand, the FL of p-PLDH films remained extremely stable after 96 h of baking without any shift in the emission wavelength, suggesting durable thermal stability of p-PLDH. The PL intensity ratios increased until they exceeded 100% after 24 h of baking at 150 °C. The weaker PL before baking can be attributed to the interaction between the absorbed water molecules and the PD chromophores within the LDH, which is possible according to theoretical and experimental investigations on some compounds with

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fluorescent chromophores[68,69]. This interaction may also be caused by the formation of localized electronic surface states[70], minimizing the optimum FL capabilities of PD. The thermal stability trend on LDH composites associated with the loss of water has also been reported in the LDH/(PVA-CdSe/ZnS) system,[55] where the FL intensity increases over the baking time.

We also incorporated p-PLDH into flexible PVA films (Figure 7c). The films of p-PLDH exhibit the homogeneous FL and bendable feature. We demonstrated that p-PLDH nanocomposites displayed enhanced thermal durability and excellent FL stability and can be doped into flexible substrates. LDH solidification can be promising for the future use of PD in solid-state optoelectronics.

4. CONCLUSIONS

We have successfully demonstrated PD solidification approaches with unquenched brightness and much improved photostability under harsh conditions and redispersiblility after solidification The synthesized nanomaterials are ideal for in vitro/in vivo bioimaging applications with minimal cytotoxicity as that of PDs. Moreover, the superior thermal durability of PLDH is noteworthy for potential applications in other areas such as light-emitting electronics. A physical mixing method provides an extremely simple and time- and energy-effective process (10 min blending) with a performance comparable to those of other synthetic methods designed in this study. The robust PL performance in p-PLDH is due to the LDH sandwich assemblies and PD deshaping. The facile, one-pot combination of LDH with various fluorescent PDs (from blue to near-IR) will create new avenues of robust PD−LDH hybrids in optical devices and biorelated technologies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsanm.7b00029.

Photoluminescent spectra, XRD data, SEM images, size distribution analysis, optical measurement data, and time-resolved fluorescent decay experiments (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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