Supporting Information

Photostable and Luminescent ZnO Films: Syntheses and Application as Fluorescence Resonance Energy Transfer Donors

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Experimental

Materials and Reagents. All chemicals were purchased from commercial sources (Alfa Aesar, Gelest, Lancaster and Sigma-Aldrich) unless otherwise specified, and were used without further purification. TMR dye and its derivatives were purchased from Invitrogen. The ZnO nanocrystals were synthesized as reported previously.[1] The NH_2-ZnO nanocrystals (~ 30 mg) were dissolved in 10 mL of deionized water. The stock solution was then filtered with a 0.2-µm membrane syringe filter prior to use. The concentration of NH_2-ZnO nanocrystals solution was quantified by UV-visible spectrometry at a wavelength of 330 nm. NH_2-ZnO nanocrystals solution should be diluted to the desired concentration right before the experiment since it was most stable at ~ 3 mg/mL.

Synthesis of Silane-Functionalized ZnO Nanocrystals. The ZnO nanocrystals were synthesized in a similar manner as the procedures reported.[1] Zinc acetate (220 mg) was dissolved in ethanol (20 mL) in an Erlenmeyer flask, and oleic acid (70 µL) was added. In a separate flask, TMAH (360 mg) was dissolved in ethanol (5 mL). Both solutions were heated to boiling and mixed together quickly as a clear solution. The reaction mixture was kept boiling for exactly 2 min, and was stopped with the addition of 50 mL of ethanol. The reaction mixture was then cooled immediately in an ice bath at 0°C. ZnO precipitates were centrifuged, collected, and redissolved in toluene (10 mL). The two-stage silanization was illustrated using AEAPS as an

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[1] (a) N. R. Jana, Y. Chen and X. Peng, *Chem. Mater.*, 2004, **16**, 3931; (b) N. R. Jana, H.-h. Yu, E. Mohamed Ali, Y. Zheng and J. Y. Ying, *Chem. Commun.*, 2007, 1406.

example. 0.1 M of AEAPS solution in toluene (1 mL) and 0.1 M of tetramethylammonium hydroxide (TMAH) solution (1 mL) were added to the ZnO solution in toluene. The reaction mixture was stirred and heated to 85°C for 15 min. It was centrifuged and washed three times with toluene to remove excess oleic acid. The precipitates were redispersed in toluene. 0.1 M of TMAH solution (1 mL) was then added. The reaction mixture was heated to 85°C for 30 min. The functionalized ZnO nanocrystals were then centrifuged, collected, and dried in vacuum oven overnight. The nanocrystals (~ 30 mg) were dissolved in 10 mL of deionized water. If necessary, formic acid (1 M) was added dropwise to increase the solubility of the nanocrystals. The stock solution was then filtered with a 0.2-μm membrane syringe filter prior to use. The concentration of nanocrystalline NH_2-ZnO solution was quantified by UV-visible spectrometry at a wavelength of 330 nm. The NH_2-ZnO solution was diluted to 0.3 mg/mL prior to the experiment since it was most stable at ~ 3 mg/mL.

Optical Measurements and AFM Studies. Absorption spectra of samples were obtained at room temperature with an Agilent 8453 UV-Vis spectrometer. Luminescence spectra were collected at room temperature with a Jobin Yvon Horiba Fluorolog luminescence spectrometer. AFM was performed with a Vecco Multimode atomic force microscope.

Preparation and Cleaning of the Glass Substrate. Pyrex glass substrates were diced to the desired size (1 cm × 1 cm or 2 cm × 2 cm) with a Disco DAD3350 automatic dicing saw. The diced glass substrates were sonicated in detergent solution for 30 min. They were then rinsed with deionized water, sonicated in 1 M of aqueous NaOH solution for 30 min, sonicated in 1 M of aqueous HCl solution for 30 min, and rinsed with deionized water. After the substrates were blown dry, they were ready for spin-coating.

Spin-Coating of NH_2-ZnO Films. Spin-coating was performed on Laurell WS-400B-6NPP-LITE spin coater. For a substrate of 2 cm × 2 cm, 680 μL of aqueous NH_2-ZnO solution (0.3 mg/mL) were dripped onto the glass surface and spun at 240 rpm for 10 min. For a substrate of 1 cm × 1 cm, 120 μL of aqueous NH_2-ZnO solution (0.3 mg/mL) were dripped onto the glass surface and spun at 500 rpm for 10 min. The substrates were then dried at 40°C until all solvents were evaporated. The films were then heat treated at 110°C for 10 min. These procedures, including spin-coating speed, drying temperature, heat treatment temperature and solvent, were optimized after a number of trials. The representative experimental trials are listed in Table S1, and the optimized results are highlighted in blue. Figure S1 shows the fluorescent images of several ZnO films under different coating and drying conditions. Figure S2 illustrates the fluorescent images of 6 different 2 cm × 2 cm samples and 3 different 1 cm × 1 cm samples obtained with the same spin-coating and drying conditions. As shown in Figure S3, the fluorescence intensities of 4 ZnO films before and after heat treatment were similar under the identical measurement parameters.

Biotinylation of NH_2-ZnO Films. Spin-coated NH_2-ZnO film was immersed in 10 mM of borate buffer containing NHS-biotin solution (0.01 mg/mL) at 4°C for 6 h. The film was then removed from the solution, and rinsed twice with deionized water. The above biotinylation procedure was repeated three times.

Lifetime Measurements. ZnO films before and after attachment to TMR dyes were excited by unfocused laser beams at 400 nm (300 MW). Emission of the films at 520 nm was recorded at every nanosecond. Lifetime of the films was obtained from the equation, $\ln (I_t/I_0) = -t/\tau$, where I_t is the fluorescence intensity at time (t), I_0 is the maximum fluorescence intensity, and τ is the fluorescence lifetime.

Table S1. Spin-coating of ZnO films on glass substrates at different conditions. The films were dried at 40°C.

Substrate Size (cm × cm)	Speed (rpm)	Duration (s)	UV Irradiation	Visual Inspection
1 × 1	300	30	fluorescent	not uniform
1 × 1	380	30	fluorescent	not uniform
1 × 1	450	30	fluorescent	not uniform
1 × 1	500	30	fluorescent	more uniform
1 × 1	750	30	less fluorescent	more uniform
1 × 1	1000	30	non-fluorescent	no film formation
1 × 1	500	60	fluorescent	incomplete surface coverage
1 × 1	500	120	fluorescent	incomplete surface coverage
1 × 1	500	300	fluorescent	improved surface coverage
1 × 1	500	600	fluorescent	full surface coverage
2 × 2	500	600	fluorescent	circular pattern
2 × 2	240	600	fluorescent	full surface coverage
2 × 2	120	600	fluorescent	incomplete surface coverage

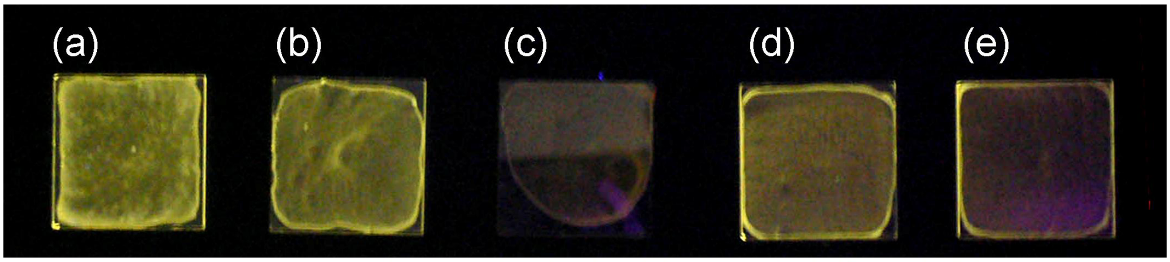

Figure S1. ZnO films prepared by different methods: (a) drip-coated and dried at room temperature, (b) spin-coated and dried at room temperature, (c) spin-coated and dried at 160°C, (d) drip-coated and dried at 40°C, and (e) spin-coated and dried at 40°C.

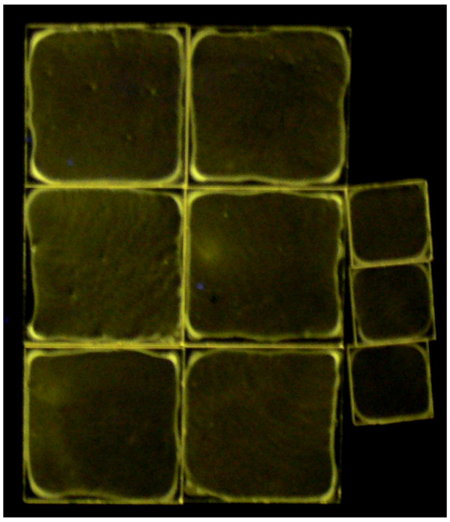

Figure S2. ZnO films on 2 cm × 2 cm and 1 cm × 1 cm substrates obtained with the optimized film fabrication conditions.

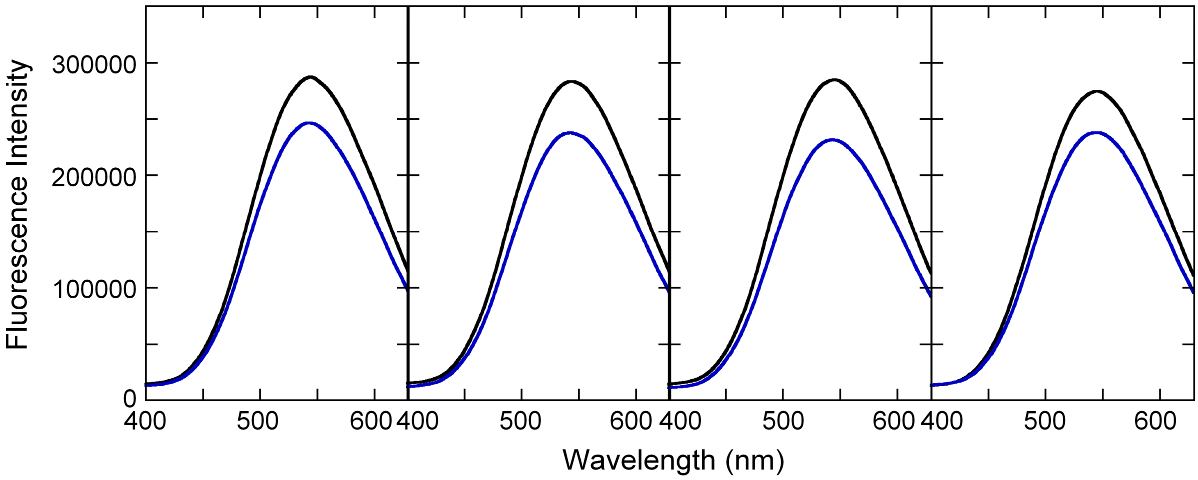

Figure S3. Fluorescence spectra of 4 different ZnO films prepared by spin-coating and drying at 40°C before (—) and after (—) heat treatment at 110°C.

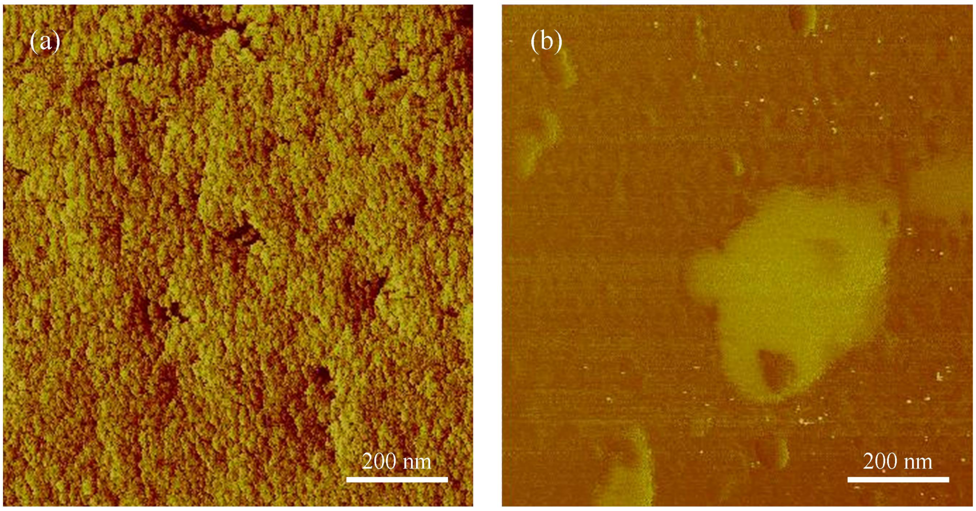

Figure S4. AFM images of ZnO films spin-coated from nanocrystalline ZnO solution in (a) water and (b) methanol.

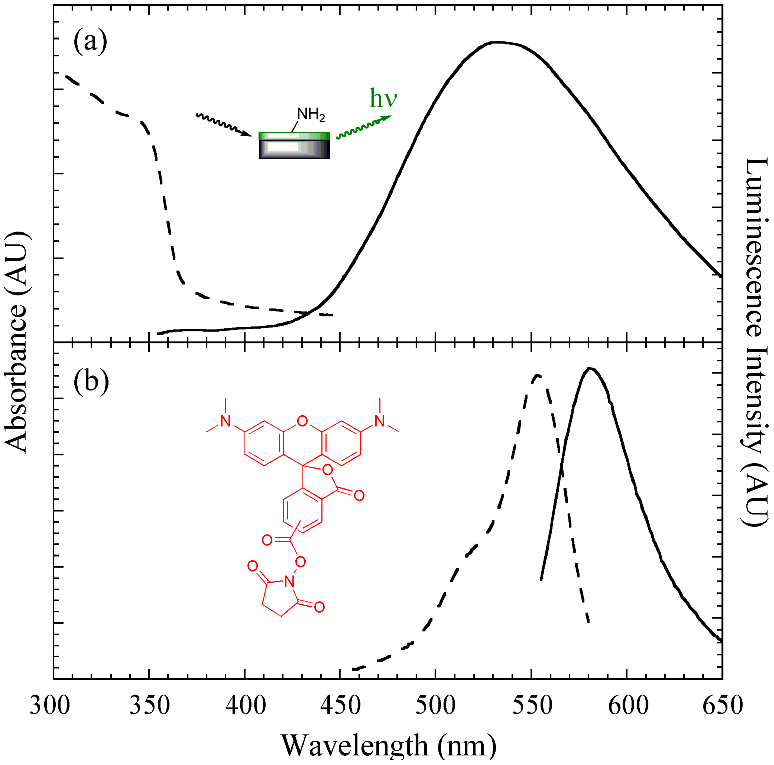

Figure S5. Absorption spectra (---) and emission spectra (—) of (a) as-coated luminescent ZnO film (excitation at 345 nm), and (b) TMR-succinimidyl ester dye (excited at 545 nm).

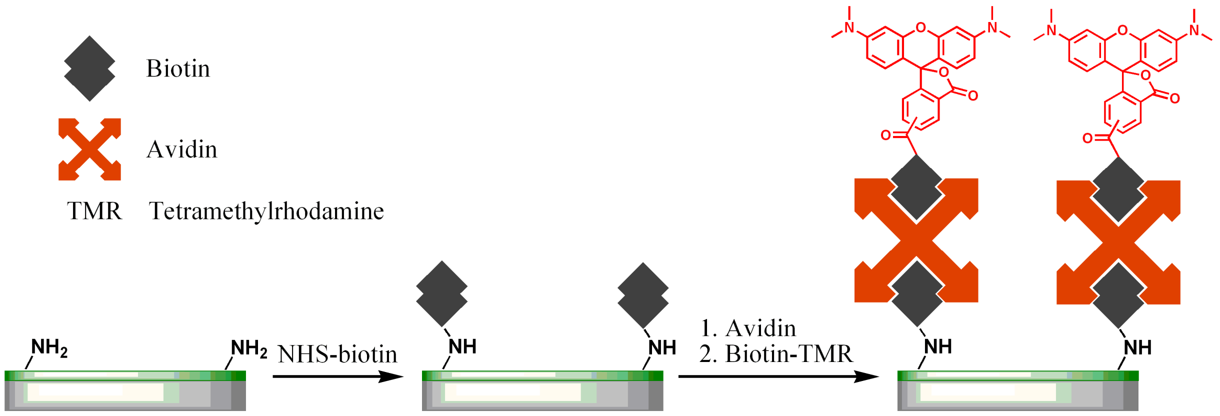

Figure S6. Schematic of ZnO film surface functionalization, and assembly of TMR-biotin/avidin/biotin-ZnO system for FRET.

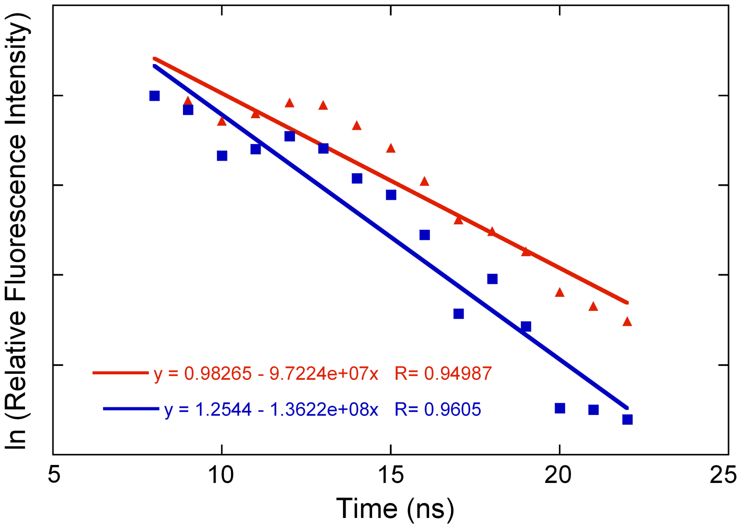

Figure S7. Lifetime measurements of luminescent ZnO films before (—) and after (—) the attachment of TMR dye. The excitation wavelength was 400 nm, and the luminescence intensity at 520 nm was recorded.