Photostable and luminescent ZnO films: synthesis and application as fluorescence resonance energy transfer donors†

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Photostable and luminescent ZnO films are effectively engineered from the corresponding nanocrystalline ZnO solutions, and they successfully demonstrated their capability as fluorescence resonance energy transfer (FRET) donors.

Fluorescence resonance energy transfer (FRET) serves as a popular signal transduction mechanism to develop biosensing systems and bioassays for proteins, peptides, nucleic acids and small molecules.[1–4] Although organic luminophores are commonly applied in commercially available FRET-based bioassays,[5] they suffer from high cost and susceptibility to photobleaching. Recent advances in luminescent colloidal nanocrystals[6] have expanded the range of luminophore candidates for FRET-based applications. Most reports are focused on cadmium-containing nanocrystals (CdSe or CdTe) due to their controllable emissions and narrow bandwidths.[7] However, the intrinsic toxicity of cadmium is of great concern in both synthesis and application.[8] In contrast, ZnO-based materials have been mass-produced and utilized in a wide variety of applications without toxicity issues.[9] They are therefore of high interest for biosensor and bioassay applications.

We previously reported the synthesis of a luminescent nanocrystalline ZnO solution in water by coating the luminescent ZnO core with a thin silane shell.[10] However, the concentration-dependent stability of our nanocrystalline ZnO solution restricted its application. In addition, once the nanocrystals were dried and stored over an extended period of time, they became insoluble in water. These limitations reduced the materials' flexibility in bio-related applications. Although recent studies on poly(ethylene glycol methyl ether)-grafted ZnO nanoparticles demonstrated improved stability,[11] the lack of surface functional groups disallowed further conjugation. Inspired by previous studies of colored,[12] magnetic,[13] and fluorescent[14] ZnO films with great stability, we have fabricated fluorescent ZnO films with functional groups suitable for conjugation to improve the stability and avoid the solubility issues related to the nanocrystalline ZnO solution.

The schematic of engineering luminescent ZnO film is illustrated in Fig. 1(a). We first prepared ZnO nanocrystals protected by *N*-(2-aminoethyl)aminopropyltrimethoxysilane (AEAPS) (abbreviated as NH_2-ZnO nanocrystals). The surface amino groups allowed these nanocrystals to be bioconjugated or functionalized, and we have previously found that AEAPS protection provided ZnO nanocrystals with the best stability.[10] Aqueous solution of luminescent NH_2-ZnO nanocrystals was spin-coated onto glass substrates at a low speed due to the high viscosity of the solution. Higher spin-coating speed resulted in the splashing of aqueous solutions, and films with ZnO islands were formed. We have examined several film-casting methods and found that spin-coating at a low speed (240 rpm for a 2 cm × 2 cm glass substrate) over an extended period (10 min) represented the optimal conditions.[15] The most uniform films were obtained when the coatings were dried slowly (in ~1 h) at 40 °C. When the films were dried at room temperature, they displayed inhomogeneity with a circular pattern. Drying at temperatures above 40 °C led to films with reduced luminescence intensity. The fluorescence intensity of ZnO nanocrystals has been known to be influenced by adsorbed oxygen species, acetates, OH groups, and

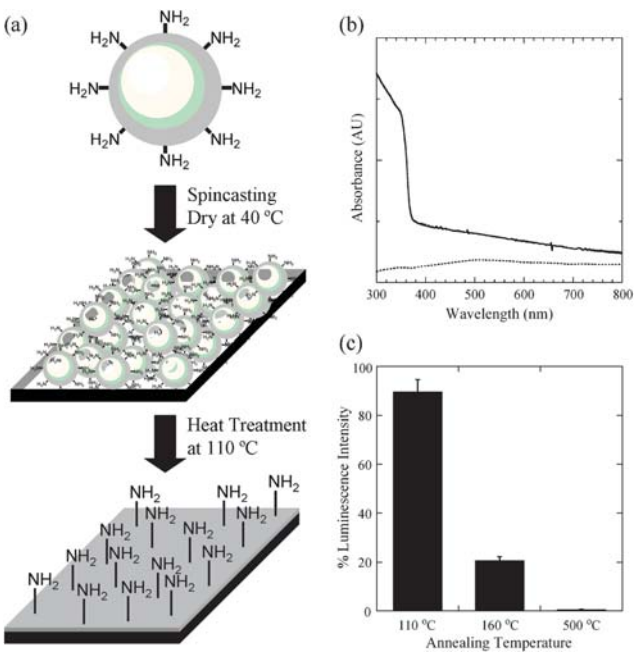

Fig. 1 (a) Engineering of NH_2-ZnO thin film from luminescent nanocrystalline NH_2-ZnO solution. (b) Absorption spectra of the heat treated (—) and untreated (···) ZnO films after sonication in water for 2 min. (c) Percentage of the luminescence intensity remaining after heat treating the spin-coated ZnO film at different temperatures.

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† Electronic supplementary information (ESI) available: Experimental details on optimization of film fabrication process, fluorescence spectra before and after sample heat treatment, AFM images, and absorption and emission spectra of ZnO films and TMR. See DOI: 10.1039/b809071h

alcohols. Given the molecular structure of our nanocrystals,[16] we attributed the reduced luminescence intensity upon heating at elevated temperature to the loss of adsorbed oxygen species and alcohols. The films derived by our spin-coating and drying protocol displayed consistent fluorescence intensity with a standard deviation of less than 10%. We also observed that the NH_2-ZnO film was redissolved after sonication in water. To increase the adhesion between the nanocrystalline NH_2-ZnO film and the glass substrate, the coated substrate was heat treated at 110 °C for 10 min after the films were dried. Fig. 1(b) shows that the NH_2-ZnO film became resistant to dissolution after this heat treatment. This was likely due to the covalent bond formation between the unreacted surface $-OCH_3$ groups of the thin siloxane shell and the surface silanol groups of the glass substrate.[17] The temperature and duration of this heat treatment were critical, and the experimental conditions were optimized for the nanocrystalline ZnO solution after many trials. Fig. 1(c) shows that >98% luminescence was quenched when the film was heated to 500 °C. 90% of the luminescence intensity was retained when the heat treatment temperature was lowered to 110 °C. Heat treatment for more than 10 min resulted in more luminescence decay, similar to that observed when the film was dried at elevated temperatures. Alternative procedures for assembly of functionalized ZnO films through silanization after film deposition are also feasible.[14] However, we found it difficult to obtain consistent results by such protocols since the silanization step required reflux in toluene for several hours, leading to the fluorescence decay of the ZnO film. Spin coating from ethanolic or methanolic solution also resulted in <50% coverage of the substrate surface.

Atomic force microscopy (AFM) showed that the spin-coated NH_2-ZnO film has a high uniformity (Fig. S4 of Electronic Supplementary Information (ESI)†) with full coverage of the substrate surface. ZnO solution and film displayed almost identical spectroscopic features. The NH_2-ZnO film showed a broad absorption in the UV region, which decreased sharply above 350 nm. The film's emission peak shifted slightly to 537 nm, in contrast to 545 nm for the solution. Fluorescamine experiments confirmed the existence of NH_2 groups on the surface of ZnO film.[18] We previously observed a 20% reduction in the luminescence peak intensity of nanocrystalline NH_2-ZnO solution after exposure to UV light, and aggregate formation in the solution after extended exposure to sunlight.[10] In contrast, NH_2-ZnO films displayed robustness against UV irradiation. The luminescence intensity at 545 nm increased by >20% when the film was continuously excited at 345 nm for 10 min (Fig. 2(a)). The increase in luminescence was due to the lattice perfection of ZnO under continuous irradiation.[19] Stability of the films was then examined by storing the films at 4 °C without covering them (Fig. 2(b)). Similar results to Fig. 2(a) were observed over an extended period of time. Luminescence of ZnO film increased from lattice perfection, whereas luminescence of ZnO solution (0.4 mg mL^{-1}) was reduced due to photobleaching. We observed total luminescence quenching within a week if the solution was diluted ten times (0.03 mg mL^{-1}).

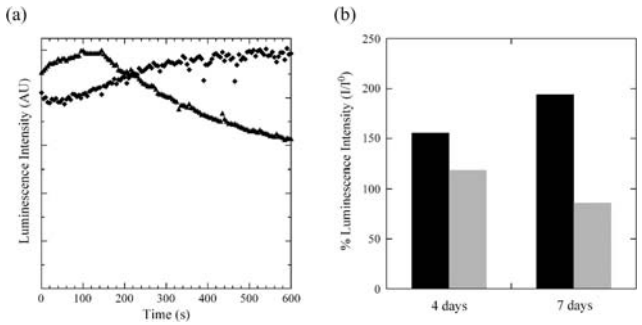

Fig. 2 (a) Kinetic luminescence measurements of nanocrystalline ZnO (▲) solution and (◆) film. The excitation and emission wavelengths were 345 nm and 545 nm, respectively. (b) Fluorescence stability of (■) ZnO film and (■) ZnO solution (0.4 mg mL^{-1}) at 4 °C.

To demonstrate the feasibility of applying luminescent ZnO film for FRET, the NH_2 groups were reacted with succinimidyl ester-activated tetramethylrhodamine (TMR) dye (Fig. 3(a)). TMR was selected as the FRET acceptor for the broad spectral overlap between its absorption spectrum and the emission spectrum of NH_2-ZnO film (see Fig. S5 of ESI†). Although the absorption from the grafted TMR group could not be observed due to the low concentration of the surface NH_2 groups, a fluorescamine test confirmed the reduced number of free amino groups. Excitation of the TMR-grafted NH_2-ZnO film at 345 nm (ZnO film's excitation wavelength) clearly resulted in a 580-nm emission peak of TMR dye besides the original 537-nm emission peak of ZnO film. When excited at 345 nm, TMR dye only displayed 20% of the emission intensity observed when excited at 545 nm (Fig. 3(c)). Once the dye was grafted onto the ZnO film, TMR emission intensity from 345-nm excitation doubled that observed from 545-nm

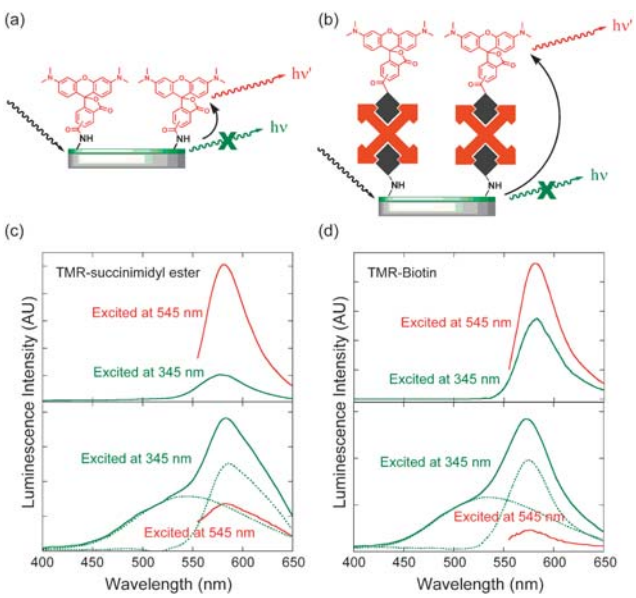

Fig. 3 Schematic of FRET between luminescent ZnO film and TMR through (a) direct attachment and (b) biotin/avidin/biotin assembly. (c) Emission spectra of TMR-succinimidyl ester dye (top), and TMR-grafted ZnO film as shown in (a) (bottom). (d) Emission spectra of TMR–biotin dye (top), and assembled TMR–biotin/avidin/biotin–ZnO film as shown in (b) (bottom). Excitation wavelengths were 345 nm (—) and 545 nm (—). The spectra excited at 345 nm were mathematically fitted from emissions of ZnO films and TMR dyes (···).

excitation. This amplified TMR emission from 345-nm excitation confirmed the energy transfer from the luminescent ZnO film to the TMR dye grafted on its surface. The efficiency of energy transfer was estimated to be ~25% based on the lifetime measurements of fluorescence intensity of ZnO film before and after the attachment of TMR acceptor dye. The number was smaller as compared to the estimation from fluorescence intensity of ZnO films (~60%). This was most likely due to the energy release from non-radiative pathways when the donor–acceptor pair was formed.

Upon establishing FRET from the direct TMR-grafted ZnO film, the same concept was demonstrated in bioassemblies utilizing the biotin–avidin platform (see Fig. S6 of ESI†). First, we functionalized the surface of NH_2-ZnO film with *N*-hydroxysuccinimide–biotin (NHS-biotin) by immersing the film in 10 mM of borate buffer containing 0.01 mg mL^{-1} of NHS-biotin. The immersion was repeated three times in freshly prepared NHS-biotin solution due to the short half-life of NHS-biotin in solution. A fluorescamine test determined that 70% of the amino groups were bioconjugated with biotin. Compared to the control experiment (ZnO film in borate buffer without NHS-biotin), luminescence intensity of the biotinylated ZnO film was 50% lower. The film was subsequently immersed in avidin solution and TMR-tagged biotin solution to form the desired assemblies shown in Fig. 3(b).

The assembled TMR–biotin/avidin/biotin–ZnO was subjected to UV irradiation at 345 nm. The emission spectra clearly displayed reduced luminescence from the ZnO film, and enhanced luminescence from the TMR dye. Excitation of the luminescent ZnO film resulted in the major emission from the TMR dye instead of the ZnO film (see Fig. 3(d)) due to energy transfer from ZnO to TMR. The luminescence intensity from the TMR dye was four times greater when the TMR–biotin/avidin/biotin–ZnO assembly was excited at 345 nm *vs.* at 545 nm. This was in contrast to the original TMR-tagged biotin solution, which exhibited only 60% luminescence intensity when excited at 345 nm *vs.* at 545 nm. ZnO served as the antenna for light harvesting in the TMR–biotin/avidin/biotin–ZnO assembly, and transferred the energy to the surface dye. Given the higher concentration of ZnO *vs.* TMR and the efficient energy transfer from the former to the latter, greater luminescence from the TMR dye was achieved at the excitation wavelength of ZnO (345 nm) than at the excitation wavelength of TMR (545 nm).

In conclusion, we have successfully engineered photostable and luminescent ZnO films. The key steps of the process include slow drying at a low temperature, followed by heat treatment at 110 °C. Coupling this ZnO film with bioassemblies such as biotin/avidin/biotin, the luminescent ZnO film serves as a FRET donor that provides successful energy transfer to the acceptor dye (TMR). With this approach, novel target-specific bioassays can be designed for biosensor applications.

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