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Supporting Information

Aggregation-Enhanced Fluorescence in Organically Modified Silica Nanoparticles: A Novel Approach toward High-Signal-Output Nanoprobes for Two-Photon Fluorescence Bioimaging

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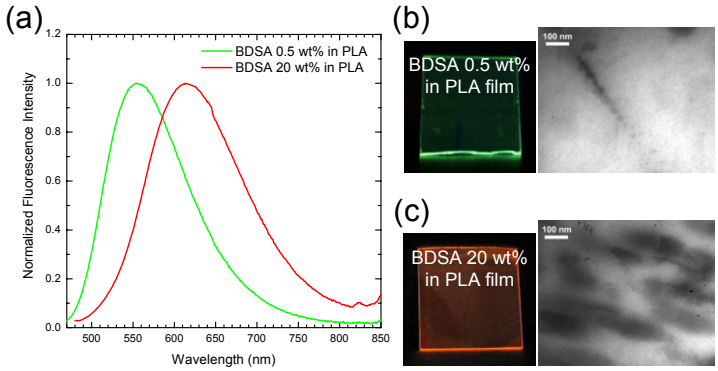

Figure S1. Evidence for phase-separated nanoaggregation of BDSA in polymer matrix at high loading. (a) Fluorescence spectra of molecular dispersion (0.5 wt%) and of nanoaggregates (20 wt%) of BDSA in poly(L-lactide) (PLA) spun films. (b, c) Fluorescence and transmission electron microscopic (TEM) images of the corresponding films. Dark areas in the TEM image of the 20-wt% loaded film (c) indicate domains of the BDSA aggregates. Typically, at lower concentrations, BDSA emits blue-shifted, greenish fluorescence, because it exists in the molecularly dispersed frozen monomer state, with π-conjugation limited by a distorted geometry. At concentrations high enough to induce phase separation from the polymer matrix, BDSA forms fluorescent aggregates with red-shifted orange emission, owing to the stacking-induced planarization of π-conjugation.

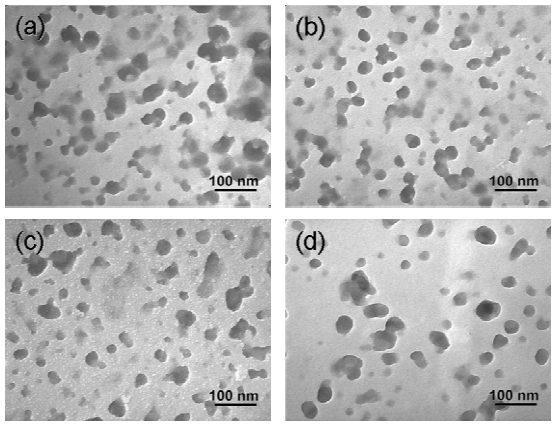

Figure S2. Representative transmission electron microscopic (TEM) images of ORMOSIL nanoparticles encapsulating the BDSA aggregates. The BDSA loading [BDSA/VTES] and the mean diameters are (a) 10 wt%,

28.9±8.7 nm, (b) 20 wt%, 27.1±6.7 nm, (c) 30 wt%, 26.7±7.8 nm, and (d) 40 wt%, 27.4±7.8 nm, respectively. In the images, it seems that some parts of particle population have been interconnected to form bigger clusters, which leads to broad size distributions.

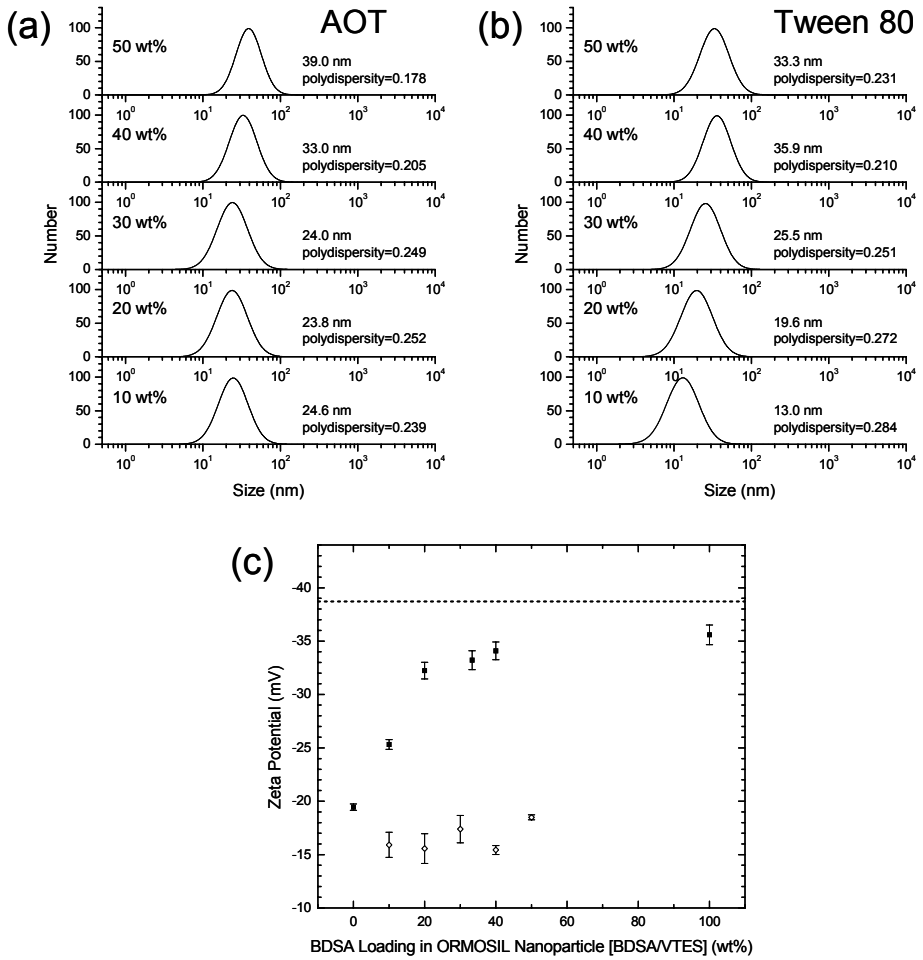

Figure S3. Effects of BDSA loading ([BDSA/VTES] by weight) on the dispersion properties of the composite nanoparticles in deionized water at pH 7.0, after removal of anionic (AOT) or nonionic (Tween 80) surfactant used for particle preparation. (a, b) Number-averaged lognormal size distribution of ORMOSIL nanoparticles, obtained by dynamic light scattering (DLS) (c) The ζ potential of ORMOSIL nanoparticles prepared using AOT (solid square) and Tween 80 (open diamond). The dashed horizontal line indicates the value of the BDSA-alone nanocrystal dispersion in deionized water at pH 7.0.

To explain the ζ potential behavior, a control set of composite nanoparticles with a similar size distribution (Figure S3b) were prepared under the same condition except for using a non-ionic surfactant (Tween 80), instead of AOT. It is unambiguous that non-ionic Tween 80, which is known to be difficult to completely remove from the particle surface, would have no significant effect on the ζ potential, irrespective of the residual amount, because it

has no intrinsic charge. As shown in Figure S3c, the Tween 80 case did not show the ζ potential modulation depending on the BDSA loading, observed for the AOT case: the ζ potential of the resulting nanoparticles is less negative and not notably modulated by the BDSA loading, in the presence of the OH^- ion and the residual Tween 80 at pH 7.0. This suggests that the preferentially adsorbed anion, in the AOT case, is not OH^-, but AOT which results in the observed ζ potential behavior in Figure S3c. The more negative ζ potential with the higher BDSA loading indicates that the increase in the surface hydrophobicity raises the residual amount of negatively charged AOT by promoting the interaction between the particle surface and the hydrophobic part of AOT.

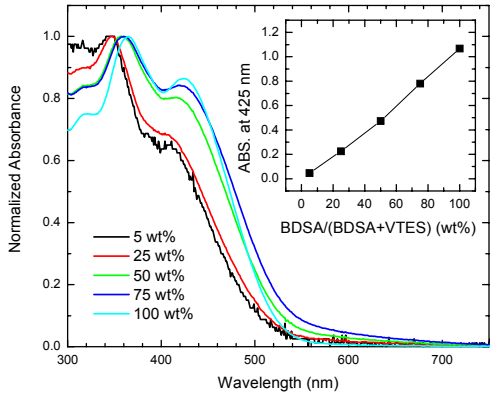

Figure S4. Normalized absorption spectra of BDSA/ORMOSIL composite nanoparticles with varying BDSA composition against total weight (BDSA/[BDSA+VTES] in wt%). The initial feed weights (BDSA+VTES) for the particle preparation were kept the same for all composition samples for quantitative comparison. The 100 wt% sample means the BDSA-alone nanocrystal dispersion. The inset shows the linear dependence of absorption at 425 nm, on the BDSA composition.

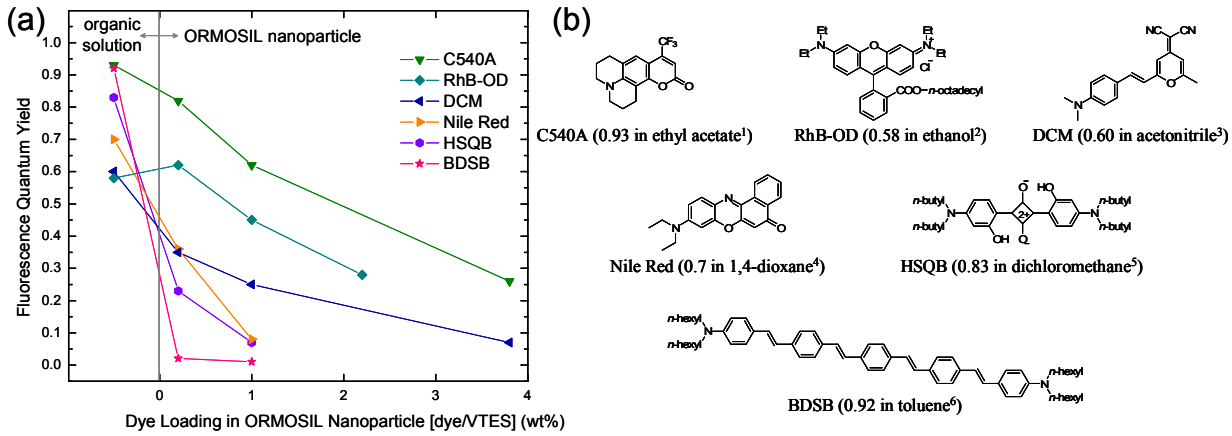

Figure S5. (a) Fluorescence quantum yields of common dyes in ORMOSIL nanoparticles, depending on dye loading. (b) Structures of common dyes examined, and their fluorescence quantum yields in solution, which are

plotted in the left part of (a). The quantum yields of nanoparticles were estimated using each dye solution noted in (b) as a reference.

References

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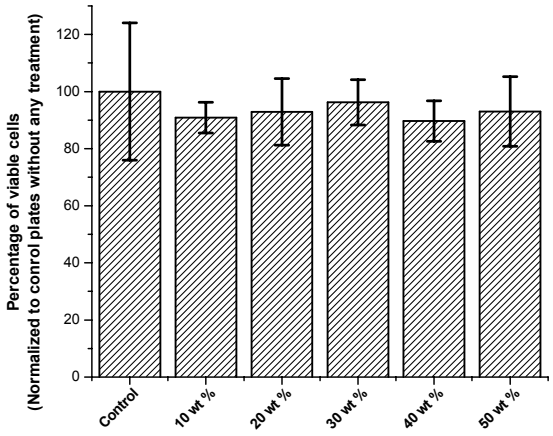
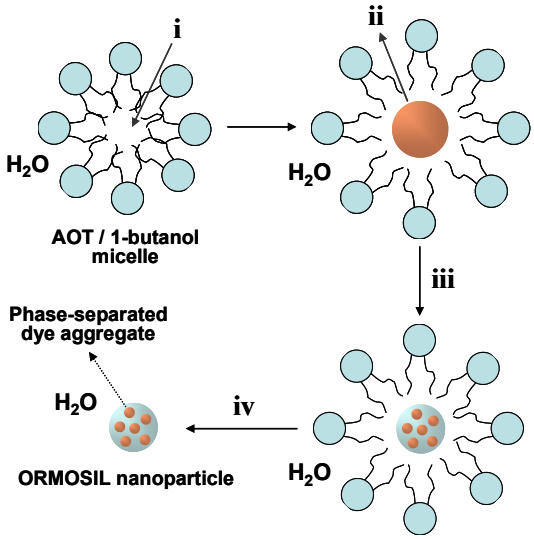


Figure S6. Percentage of cell survival of HeLa cells, after treatment with the composite nanoparticles for 12 hours, determined using CellTiter-Glo^{TM} Luminescent Cell Viability Assay (Promega Corporation, Madison, WI), which generates luminescent signals, based on the quantification of the cellular ATP levels. Hela cells in a medium supplemented with fetal bovine serum (10%) in a 96-well plate (8×10^4 cells/well) were treated with nanoparticles in a similar manner as described for imaging and flow cytometry experiments. After 12 hours of incubation, cells were washed multiple times and fresh growth media was added. After another 30 minutes of incubation in a 37°C incubator, the plates were brought to room temperature, followed by treatment with CellTiter-Glo reagents as per the manufacturer's protocol. Luminescence was measured in a Biotech Synergy HT, a multilabel, multitask plate counter, to determine the number of viable cells. Each point represents the mean ± SD (bars) of at least quadruplicate and was normalized against values for control cells without any treatment with nanoparticles.



Scheme S1. Preparation scheme for the ORMOSIL nanoparticles encapsulating phase-separated dye aggregates: i. Injection of homogeneous NMP solution of prepolymerized VTES sol and BDSA into an aqueous dispersion of AOT micelles. ii. Transient emulsification of NMP solution in the nonpolar micelle interior. iii. Phase-separated dye aggregation during spontaneous coprecipitation of VTES sol and dye via depletion of NMP into the aqueous exterior, and further condensation into gel. iv. Removal of AOT by dialysis against water.