SUPPORTING INFORMATION

Peptide-Mediated Constructs of Quantum Dot Nanocomposites for Enzymatic Control of Nonradiative Energy Transfer

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Peptides: Peptides were purchased from Alamanda Polymers, AL, USA. Peptide solutions were prepared in phosphate buffer saline, and the pH of the solution was adjusted to 7.4 using 0.1 M HCl. The peptides have a purity level of 95%. The peptide solutions were kept at 4°C. The peptide solutions for isothermal titration calorimetry and circular dichroism spectroscopy were prepared in phosphate buffer saline composed of 137 mM NaCl, 2.7 mM KCl, 10 mM $NaHPO_4$, and 1.76 mM KH_2PO_4.

Nanocrystals: Water-soluble, negatively charged CdTe nanocrystals stabilized with thioglycolic acid (TGA) were synthesized following the protocol given in Shavel *et al.*[1]. First, solutions of 22.95 g/L $Cd(ClO_4)_2$ and 4.36 g/L TGA were prepared in deionized water. Two solutions were mixed and the pH of the final solution was adjusted to 12 using 1M NaOH, and the final solution was flushed with argon and kept under argon atmosphere. 0.8 g Al_2Te_3 was dissolved using 10 mL of 0.5 M H_2SO_4 solution slowly under argon gas flow in a glove box. In the meantime TGA–$Cd(ClO_4)_2$ mixture was moved into the Al_2Te_3 solution using argon as the carrier gas. After the final solution became reddish, Ar gas was turned off. The solution was kept at 100 ^{0}C with a cooler connected to the system. During synthesis, nanocrystals start to grow as the boiling starts. Therefore, as the boiling of the final solution continued,

CdTe nanocrystals at varying sizes were formed depending on the boiling time. In the very first minutes green nanocrystals at a wavelength around 560 nm were prepared, which served as the donor nanocrystals in our study. Half of the nanocrystals were removed from the flask at this state to use them as the donors in our experiments. The growth rate of nanocrystals is fast at the beginning of the reaction; in very short time interval the peak emission wavelength of nanocrystals red-shifts. However, the growth rate (and consequent shift of the peak emission wavelength) becomes slower with increasing time. In our case, it took approximately 23 hours to achieve red-emitting nanocrystals around 640 nm, which served as the acceptor nanocrystals in our experiments. The reported values correspond to the peak emission wavelengths of the nanocrystals in solution, which further red-shift by 15-20 nm in the film. To purify the nanocrystals, we evaporated 90% of the solvent in the rotary evaporator. Remaining highly concentrated nanocrystal solution was sonicated and centrifuged at 4,500 rpm for 5 minutes. The precipitate was dissolved in Milli-Q water and centrifuged for a second time at a rate of 13,000 rpm for 2 min.

Layer-by-layer (LbL) assembly: LbL assembly was carried out using a dip coater from Nima Technology, UK. The samples were dipped and pulled at a constant speed. Peptides were prepared as 1 mg/mL concentrations in PBS buffer and QDots were prepared as 3 μM concentrations. The Corning glass substrates before coating were immersed into a 1M KOH solution and rinsed with DI water to ensure that the silica surface was negatively charged.

Isothermal titration calorimetry (ITC): All of the calorimetry experiments were performed using Microcal 200 equipment (GE Healthcare, Austria). The concentration of peptides in the cell was kept ten times lower than the peptide concentration in syringe for all of the peptide pairs. The runs were carried out at 25°C and in PBS buffer at 200 rpm. The peptide concentration in syringe was kept at 163 μM and in the ITC cell peptide concentration was kept at 18.7 μM. The raw data were fitted using the

software *Origin 7* supplied along the ITC200. After each run the instrument was automatically and manually cleaned with methanol, detergent and DI water.

Circular dichroism (CD) spectroscopy: All CD spectra were obtained at 25ºC using an JASCO J 815 CD spectrometer. The CD spectrometer was previously calibrated with d-10-camphorsulfonic acid. CD measurements were taken using peptides with 25 µM concentrations dissolved in phosphate buffer (pH 7.4) in a quartz cuvette with 1 mm path length. Wavelength scans were conducted from 195 to 240 nm with phosphate buffer and solvent background subtraction. For each spectrum, three scans were averaged using 1 nm bandwidth and a scanning rate of 0.5 nm s^{-1}. Mean residue ellipticity [θ_M] was expressed in L $mol^{-1}cm^{-1}$.

Quartz crystal microbalance with dissipation monitoring (QCM-D): The *in situ* film formation during the layer-by-layer assembly was monitored using a quartz crystal microbalance Q-Sense E1 (from Q-Sense Company, Frolunda, Sweden). To mimic the silica layer we used a silica-coated sensor from Q-Sense. The assembly of each layer was carried out using a peristaltic pump at a flow rate of 10 µL/min. After each run, the sensor surface was flushed with buffer to remove non-specifically and weakly bound materials. We gathered the frequency shift and dissipation change data from QCM-D. The data collected from the instrument was fitted to a viscoelactic model[2, 3] to calculate the thickness of each layer, which is presented in Figure 4B.

Dissipation change data was fitted to the Voight type of viscoelastic model. By doing so, one can estimate the thickness of the formed peptide film on the quartz resonator surface. The equations for the viscoelastic model were expressed as follows[2, 3, 4]:

$$\Delta f \approx \frac{1}{2\pi\rho_q h_q} \hbar\rho f \left(1 + \frac{2\hbar^2\chi}{3\delta^2(1+\chi^2)}\right) \quad (1)$$

$$\Delta D \approx \frac{2\hbar^3\rho f}{3\pi f_{ro}\rho_q h_q} \frac{1}{\delta^2(1+\chi^2)} \quad (2)$$

$$\chi = \frac{\mu}{\eta f} \quad (3)$$

$$\delta = \sqrt{\frac{2\eta}{\rho f}} \quad (4)$$

where ρ_q and ρ are the density of the quartz and the adsorbed film on the resonator, respectively; h_q and h are the thickness of the quartz and the adsorbed film, respectively; f is the measured frequency of the resonator; χ is the ratio of the storage modulus (μ) to the loss modulus (η); f_{ro} is the resonance frequency of the quartz crystal; and δ is the viscous penetration depth. By taking measurements at five different overtones and using the least-squares curve fitting in *QTools* (ver. 3.0.7), we extracted the values for film thickness.

Atomic force microscopy (AFM): AFM measurements were carried out on layer-by-layer assembled samples. The samples composed of layers of peptides and QDots embedded into the peptide layers. AFM images were obtained using PSIA-XE-100E instrument. The images were taken from a 5μm x 5μm area. The recoded images were analyzed using the image analysis program *PSIA*, and a line profile of the image was plotted using this program. Later the surface coverage of the peptide films was calculated using an image-processing program *ImageJ*, available free of charge at (http://rsbweb.nih.gov/ij/).

Time-resolved photoluminescence spectroscopy (TRPL): TRPL measurements were carried out using a time-resolved photoluminescence spectrophotometer (from PicoQuant GmBH, Germany) equipped with a picosecond laser source at an excitation wavelength of 375 nm. The sample films were placed on the film holder and the emission was collected at the corresponding wavelength for the donor and acceptor QDots. Collected light , either from the donor or the acceptor, was passed through a UV filter (with a

cut-off wavelength of 400 nm) to eliminate collection of excitation source reflections from the substrate. To analyze the data we used a software program supplied along with the equipment, *FluoFit*.

Protease digestion: Protease from bovine pancreas (Sigma, St. Louis, MO, USA) was dissolved in PBS buffer (ca. 5 units per mg of solid enzyme). The pH of the protease solution was adjusted to 7.4. Peptide-based QDot composite films were immersed in protease solution for 4 minutes, and following the protease digestion the film surface was rinsed with PBS solution, and the rinsed film was dried using nitrogen. The dry film surface was prepared for TRPL measurements.

We also included a negative control experiment with enzymes other than protease to ensure that the change in the photoluminescence decay lifetime of the acceptor QDots is caused by the digestion of the peptide film with protease. In these experiments, we used lysozyme (from Sigma, 20.000 U) and β-galactosidase (from Sigma, 8 U). We carried out the control experiments under the same conditions with the protease digestion experiments. We observed that neither of lysozyme or β-galactosidase activity affects the lifetime of the acceptor QDots and does not create any change in the time-resolved photoluminescence data of the acceptors as given in Figure S1.

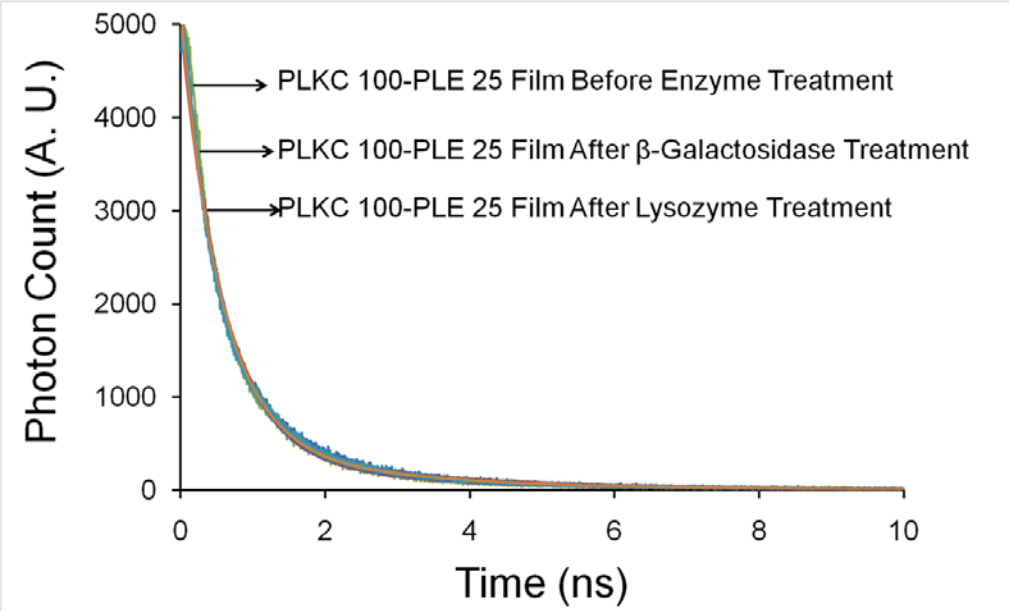

Figure S1. Time-resolved photoluminescence (TRPL) of CdTe QDots with FRET process (A), after the addition of β-galactosidase (B), and after the addition of lysozyme (C).

References

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