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Electronic Supplementary Information

Quantum Dot- Engineered M13 Virus Layer-by-layer Composite Films for Highly Selective and Sensitive Turn-on TNT Sensors

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Synthesis of Quantum dots

Materials

Oleic acid, tech. (90%), trioctylphosphine, tech. (90%, TOP), 1-octadecene, tech. (90%, ODE), oleylamine, tech. (70%), diethylzinc, bis(trimethylsilyl)sulfide (95%), and (±)-α-Lipoic acid were purchased from Aldrich. Cadmium acetate dehydrates (99.999%), and selenium shots (99.99%) were purchased from Alfa Aesar and Strem, respectively.

Synthesis of CdSe QDs

CdSe quantum dots were synthesized following the procedures previously described with a few modifications.[S1] For cadmium precursor, 0.6 mmol of cadmium acetylacetonate hydrate was dissolved in

1.8 mmol of oleic acid at 100 °C under vacuum. After the solution had cooled to room temperature, the cadmium precursor was mixed with selenium precursor. The selenium precursor was previously prepared by dissolving 3.0 mmol of selenium shots in 3.0 mL of trioctylphosphine in glovebox. 24.0 mL of octadecene and 2.0 mL of oleylamine were loaded into a 50 mL threeneck flask, and heated to 310 °C under nitrogen gas flow. At this temperature, the mixture of cadmium and selenium precursors was quickly injected into the reaction flask, and the temperature was maintained at 280 °C. The reaction mixture was kept stirred until the desired size of CdSe nanocrystals was obtained. Upon completion, the mixture was cooled to room temperature and diluted by hexanes. For purification, the product mixture was precipitated by adding excess methanol, collected by centrifugation, and redispersed in a small amount of hexanes.

Synthesis of CdSe/CdS/ZnS Core/Shell/Shell Quantum dots

For shell growth using the SILAR method[S2], CdSe quantum dots (300 nmol of particles) dissolved in hexanes were mixed with 6.0 mL of ODE and 2.52 mL of oleylamine in a 50 mL threeneck flask. The flask was then pumped down at room temperature with a mechanical pump to remove the hexanes and at 100 °C for 1 hour to remove any residual air from the system. Subsequently, the system was switched to nitrogen gas flow and the reaction mixture was further heated to 200 °C for the injections. We used precursor solutions containing only metal oxides (CdO and ZnO), and elemental sulfur. The zinc precursor solution (0.1 M) was prepared by dissolving ZnO (0.1mol) in oleic acid (10 mL) at 260 °C. The cadmium precursor solution (0.1 M) was prepared by dissolving CdO (0.1mol) in oleic acid (10 mL) at 260 °C. The sulfur precursor solution (0.1 M) was prepared by dissolving sulfur in ODE at 160 °C. All of the solutions were freshly prepared under Nitrogen-atmosphere. The Cd-, Zn-, and S-precursor solutions were kept at about 100 °C. For each shell growth, a calculated amount of a given precursor solution was injected with a syringe using standard air-free procedures. The first injection was 0.32 mL of the Cd injection solution. To monitor the reaction, aliquots were taken every 7 min after each injection that for

photoluminescence measurements. This is followed by alternating addition of S-precursors and Cd-precursors, respectively. After the complete coverage procedure of CdSe core with 2 MLs of CdS, Zn- and S-precursors was injected for 4MLs of ZnS. A period of 10 min between each addition was sufficient for the reaction to be completed. The reaction was terminated by allowing the reaction mixture to cool. The final product was diluted by hexanes followed by a methanol extraction.

Synthesis of Tertiary Amine Surface Ligands

Tertiary amine surface ligand was synthesized following the procedures previously described.[S3] Lipoic acid (20 mmol) and 1,1-carbonyldiimidazole (26 mmol) were dissolved in anhydrous chloroform (30 mL) and stirred under N_2 flow for 20 min at room temperature. The solution was added dropwise to *N*,*N*-dimethylethylenediamine (DMED) (100 mmol) in an ice bath and stirred for 2 h under N_2 gas flow. The crude product in chloroform was obtained by removing the byproducts through three extractions with 10% NaCl aqueous solution (80 mL) followed by two extractions with 10 m M NaOH aqueous solution (80 mL). It was dried with magnesium sulfate, and the solvent was removed using a rotary evaporator to obtain the yellow liquid product. The reaction yield was 95%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ [ppm]: 6.05 (br s, 1H), 3.50–3.59 (m, 1H), 3.30 (t, 2H, J = 6 Hz), 3.28 (t, 2H, J = 6 Hz), 3.05–3.20 (m, 2H), 2.38–2.49 (m, 1H) 2.38 (t, 2H, J = 6 Hz), 2.20 (s, 6H), 2.17 (t, 2H, J = 9 Hz), 1.83–1.94 (m, 1H), 1.57–1.72 (m, 4H), 1.38–1.52 (m, 2H); MS (FAB, m/z): M calculated for $C_{12}H_{24}N_2OS_2$, 276.1; $[M+H]^+$: found, 277.1.

Surface Modification of CdSe/CdS/ZnS Core/Shell/Shell Quantum dots

CdSe/CdS/ZnS (Core/Shell/Shell) QDs were ligand exchanged by tertiary amine surface ligands. Excess amount (typically more than million times of the number of QDs) of the surface ligands (oxidized form) were dissolved in PBS buffer solution. 2 equimolar sodium borohydride was added to the solution and vigorously stirred for at least 30 minutes under N_2 gas flow at room temperature. The QD solution (in chloroform) was added to the PBS buffer solution and further stirred for at least 4 hours at room temperature. The QDs were transferred from the organic layer to PBS buffer layer. To remove excess free surface ligands, the QD solution was dialyzed twice using Amicon 50 kDa M_W cutoff centrifugal filter.

Genetic Engineering of Phage and Phage Display

In order to develop major coat engineered phage for the TNT explosive, we first identified the TNT binding peptide using phage display using commercially available 12mer linear peptide library (Ph.D.™-12).[S4] In short, phage display was performed with the initial binding condition of 0.1% TBST for 30 minutes at room temperature on a rocking platform with 5 mg of target, TNT crystals. Following binding, a series of 10 wash steps were performed using the same binding buffer to remove non-specific binders. Specific binding phages were eluted with 1mL of 0.2 M Glycine-HCl [pH 2.2], 1 mg/ml BSA. The progressive screening rounds utilized increasing surfactant concentration to increase stringency of binding to TNT targets. Screening results were obtained through sequence analysis of the receptor region following each screening round. Through the phage DNA analysis, we identified the consensus TNT binding peptide (Trp-His-Trp-Gln: WHWQ) and confirmed their specificity through binding assays. In order to incorporate the consensus TNT binding peptide on major coat protein, M13 phages were genetically engineered to display specific peptide motifs on their major coat proteins (pVIII). The desired peptide sequences were inserted between the first and the sixth amino acids of the N-terminus of wild type pVIII, replacing residues 2-5 (Ala-**Glu-Gly-Asp-Asp**-Pro to Ala-(**Insert**)-Pro) as previously reported[S5] In short, in order to incorporate the most stable phage to carry consensus TNT binding peptide (WHWQ), we used the partial library design A*XWHWQXX*DP using primer: 5'-CTGCAG*NK**TGGCATTGGCAG**NNKNNK* -3' and for The primer 5'GCTGTCTTTCGCTG CAGAGG GTG3' to linearize the vector shown in italics, N = A, T, G or C). To incorporate the gene sequences, polymerase chain reaction (PCR) was performed using Phusion DNA Polymerase, two primers (insertion and linearization), and an M13KE vector with an engineered PstI site as the template. The obtained product was purified on an agarose gel, eluted by spin column purification, digested with PstI enzyme, and recircularized by an overnight ligation at 16°C with T4 DNA Ligase. The ligated DNA vector was transformed into XL1-Blue electroporation competent bacteria, and the amplified plasmid sequence was verified at the University of California, Berkeley DNA sequencing facility. The phage solution was further purified by filtration through 0.45 μm pore size membranes. To verify phage stability, DNA sequences were confirmed at each step of the amplification.

Preparation of the Quantum Dot-Phage Layer-by-Layer Assemblies

Positively charged QD solution was prepared by dissolving tertiary amine decorated QDs in MES buffer solution. Negatively charged phage solution was prepared by dissolving WHW or wild phage in MES buffer solution. A slide glass was cleaned for 4 h with sonication in piranha solution (H_2SO_4:H_2O_2 =7:3).

The glass was dipped into 2 % Poly(diallyldimethylammonium chloride) (PDDA) solution in 0.5 M NaCl for 20 min and washed with D.I. for 2 min. The substrate was dipped into the negatively charged phage solution for 20 min and washed with D.I. for 2 min. The substrate was dipped into the positively charged QD solution for 20 min and washed with D.I. for 2 min. The time profile of adsorption showed that dipping for 20 min reaches all most saturation level. Then, the substrate was dipped into the negatively charged phage solution for 20 min and washed with D.I. for 2 min. The cycle of dipping into the negative charged phage solution, washing and dipping into the positive charged QD solution was repeated up to the desired number of cycles.

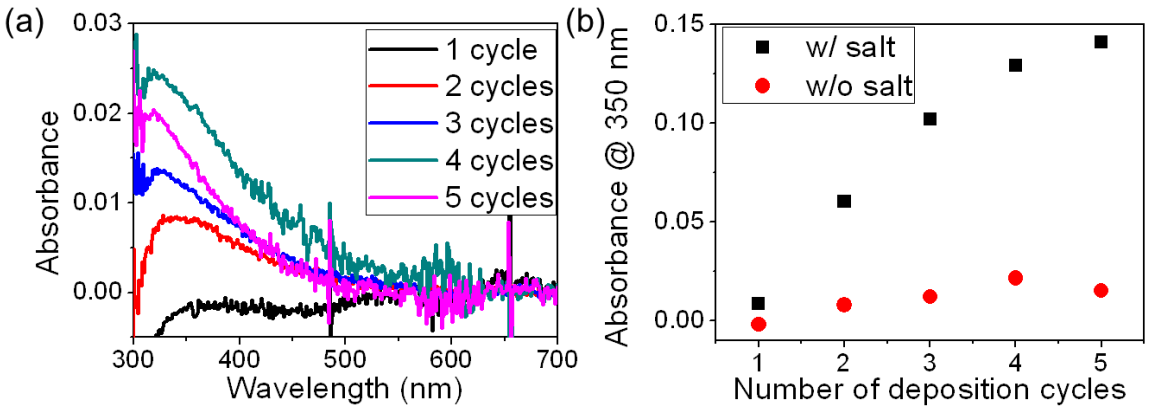

Figure S1 (a) Absorption spectra of layer-by-layer (LbL) assembled quantum dot (QD)/WHW-phage film at each deposition cycle from 1 to 5 without salt. (b) Absorbance at 350 nm of LbL assembled QD/WHW-phage film at each deposition cycle without salt (red) and with salt of 100 nM NaCl (black).

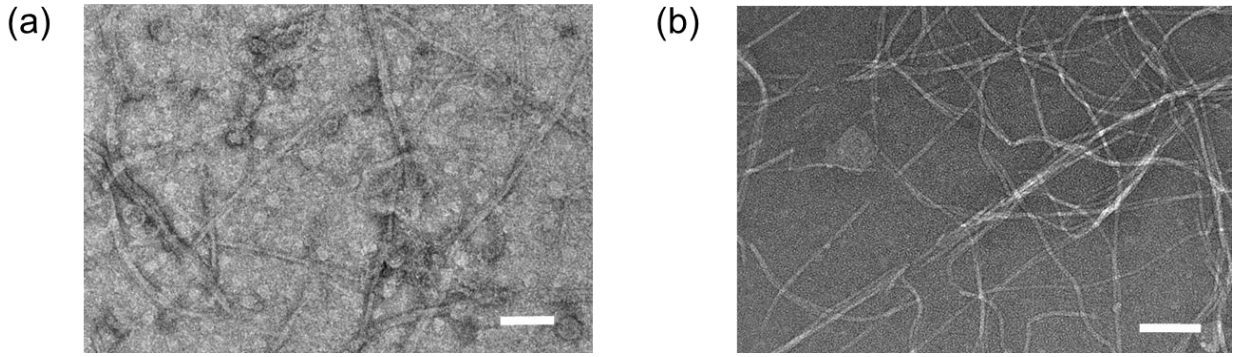

Figure S2. TEM images of (a) as-prepared WHW phage and (b) as-prepared wild phage. Scale bar: 100 nm.

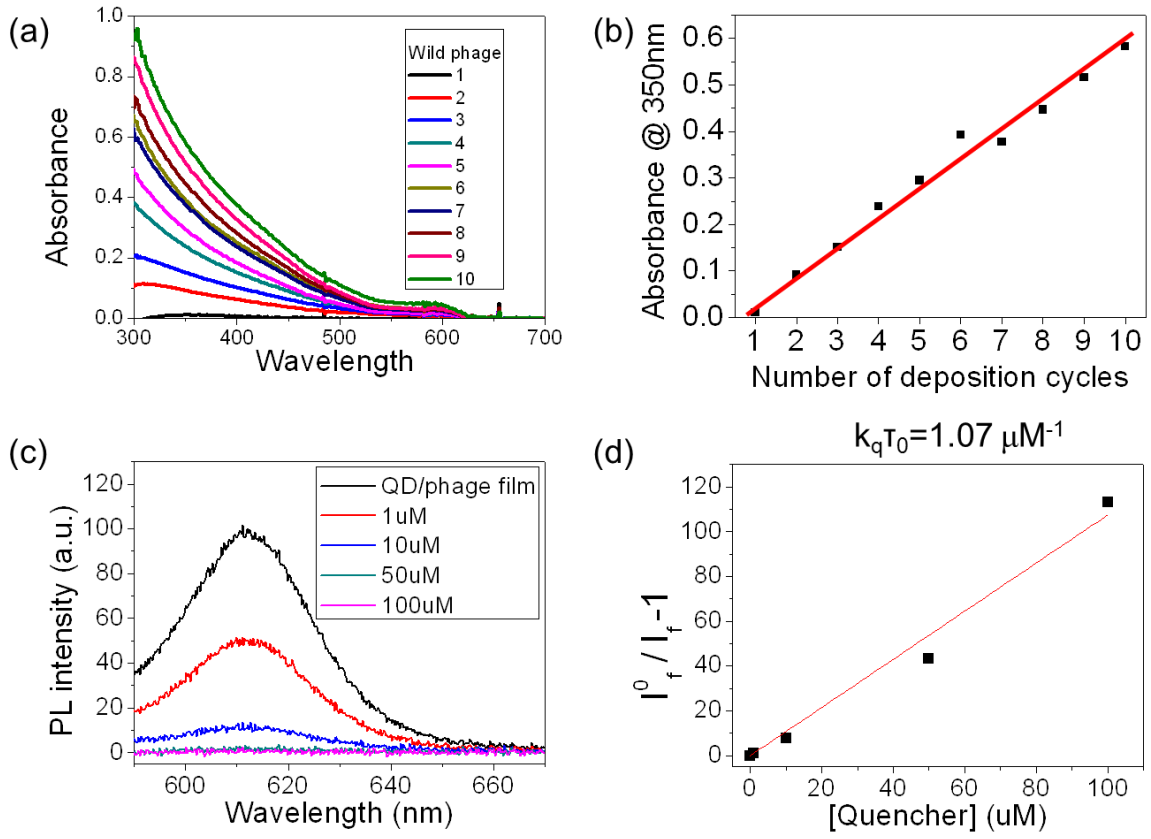

Figure S3. (a) Absorption spectra of layer-by-layer (LbL) assembled quantum dot (QD)/wild-phages film at each deposition cycle from 1 to 10. (b) Absorbance at 350 nm of the LbL assembled QD/wild-phage film at each deposition cycle. (c) The photoluminescence spectra of LbL assembled QD/wild-phages film after incorporation of the BHQ quencher of different concentrations. (d) The Stern-Volmer fitting for the plot (c).

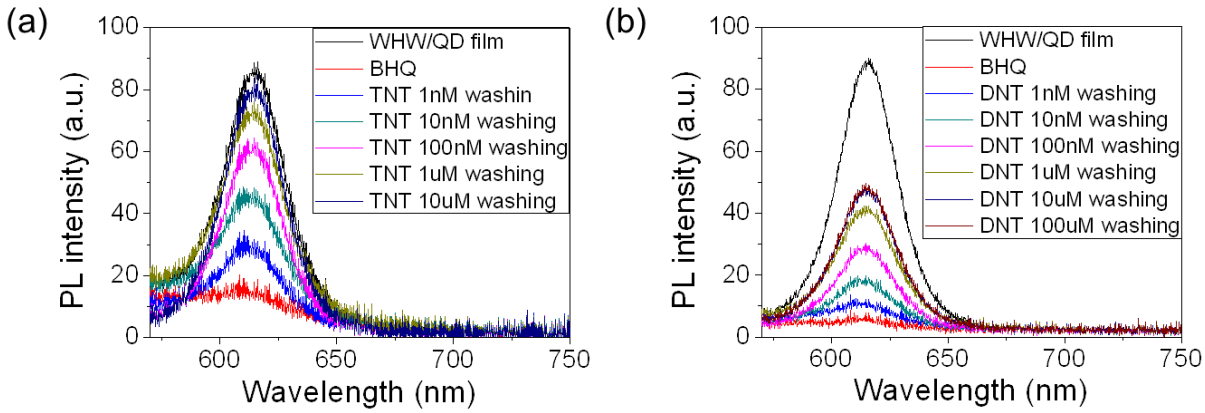

Figure S4. The photoluminescence spectra of layer-by-layer assembled quantum dot/WHW-phage film before and after the incorporation of the BHQ quencher and after the incubation in TNT (a) or DNT (b) aqueous solutions of the various concentrations.

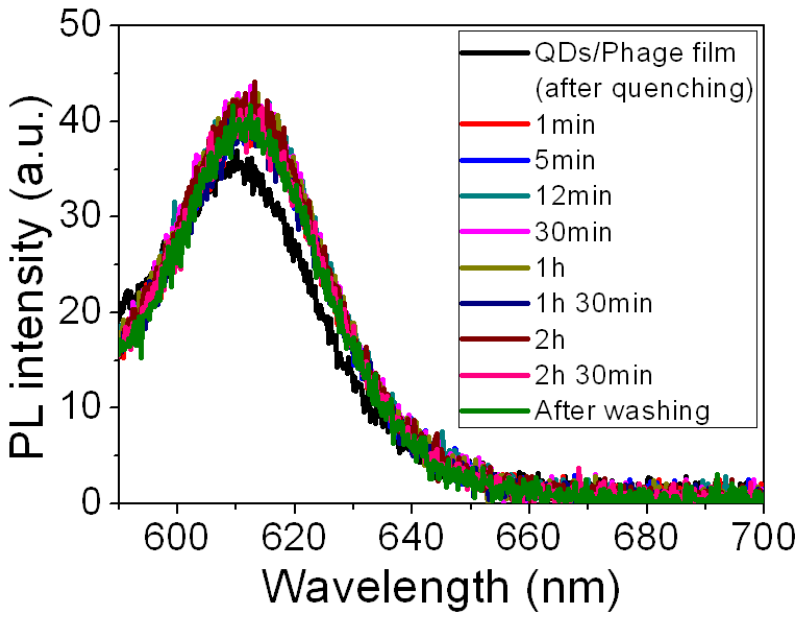

Figure S5. The photoluminescence spectra of layer-by-layer assembled quantum dot/wild-phage control film before and after the incorporation of the BHQ quencher and after the incubation in 220 μM TNT aqueous solution for various incubation times.

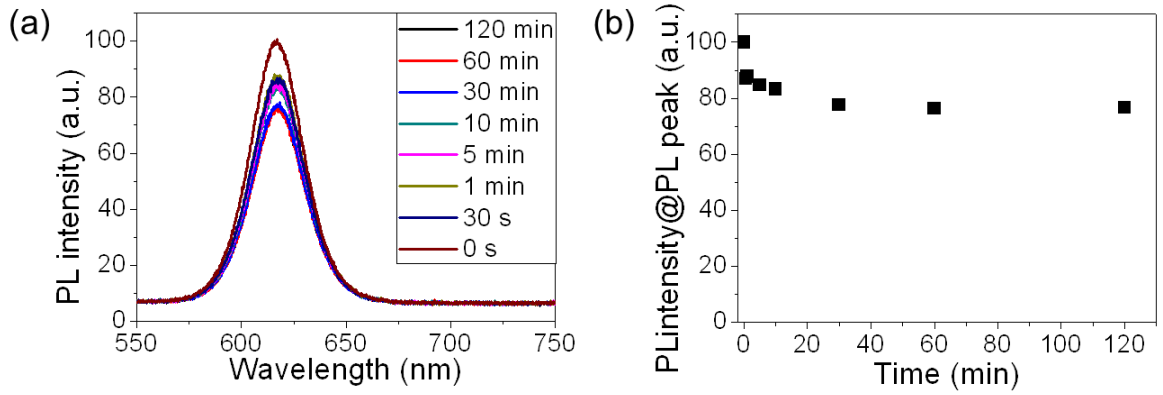

Figure S6. (a) Temporal evolution of the photoluminescence (PL) spectrum for layer-by-layer assembled quantum dot/wild-phage film of which quencher incorporation process has been omitted. The film was incubated in 220 μM of TNT solution. (b) PL intensity plot for the spectra shown in (a).

Supporting References:

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