

## Electronic Supplementary Information

### **A minimized designer protein for facile biofabrication of ZnS:Mn immuno-quantum dots**

Weibin Zhou, Brian Swift and François Baneyx\*

Department of Chemical Engineering, University of Washington,  
Seattle, WA 98195

## **MATERIALS AND METHODS**

### **DNA manipulations**

Plasmid pBB-CT43, which encodes a fusion protein between a tandem repeat antibody-binding domains from *S. aureus* protein A (BB segment) and the Zn-binding peptide CT43 under transcriptional control of the T7 promoter, was constructed as follows. Two phosphorylated primers, 5'-CTAGGGGTGGCGGAGGTAGCGCGGGTGATAGTAGTGGGGTGGACAGTCGCAGTGTTTAATGAC-3' and 5'TTAAGTCATTAACAACACTGCGACTGTCCACCCCACTACTATCACCCGCGCTACCTCCGCCACCC-3', were annealed by boiling in a water bath for 10 min and air cooling. Backbone DNA was amplified from plasmid pBB-Trx::CT43<sup>1</sup> using primers 5'-GCGTCGACCTTAAGTAATCGTACAGGGTAGT-3' and 5'-GGTTTCCGGGGATCCTCGCTATTTT-3' to introduce *Avr*II and *Af*III sites at 5' and 3' ends, respectively. Hybridized primers and backbone were ligated at a 5:1 ratio. Construct integrity was verified by sequencing.

### **Protein purification and nanocrystal synthesis**

BB-TrxA::CT43 was purified as previously described.<sup>1</sup> BB-CT43 was purified by a similar procedure. Briefly, BL21(DE3) cells harboring pBB-CT43 were grown at 37°C in LB medium supplemented with 34 µg/mL chloramphenicol to  $A_{600} \approx 0.4$  and protein expression was induced by addition of 0.4 mM IPTG. After 4h incubation at 37°C, cells were sedimented by centrifugation at 3,500g for 10 min, resuspended in 20 mM Tris-HCl, pH 7.5, 2.5 mM EDTA, 1 mM PMSF to  $A_{600} \approx 50$ , and disrupted with a French press. Lysates were clarified by centrifugation at 14,000g for 10 min and supernatants were loaded onto a Q Sepharose FF column (6.0 x 1.0 cm inner diameter) equilibrated in 20 mM Tris-HCl, pH 7.5. Contaminants were removed by washing with 20 mL of the same buffer at 1 mL/min. Bound proteins were eluted over 1h using a 0 to 250 mM NaCl linear gradient. Fractions containing pure BB-CT43

(eluting at  $\approx 100$  mM NaCl) were pooled and desalted by dialysis against 20 mM Tris-HCl, pH 7.5 using 3 kDa-cutoff SnakeSkin dialysis tubing (Thermo Scientific). Protein-capped Mn-doped ZnS nanocrystals were synthesized as previously described<sup>2</sup> using 5  $\mu$ M of purified BB-CT43 or BB-TrxA::CT43 and stored at 4°C, a temperature at which they are stable for months.

### **Agarose gel electrophoresis**

ZnS:Mn quantum dots prepared with BB-CT43- or BB-TrxA::CT43 were concentrated  $\approx 4$  times by centrifugation at 4,000 g using an Amicon Ultra-4 centrifugal filter (MWCO 3kDa, Millipore). The retentate was assayed for protein concentration (assumed to be quantitatively bound to nanocrystals) using a Bradford assay (Pierce). The typical concentration of fusion protein was 0.2 mg/mL (7.5  $\mu$ M). Human IgG purified immunoglobulin (Sigma) was dissolved to a 2 mg/mL (13.3  $\mu$ M) concentration in buffer A (100 mM Tris-HCl, pH 7.5, 150 mM NaCl). ZnS:Mn nanocrystals (30  $\mu$ L) were mixed with IgG to a molar ratio of IgG to BB-CT43 (or BB-TrxA::CT43) of 1 to 5 and the final volume was adjusted to 60  $\mu$ L with buffer A. After 1h incubation at 4°C with slow shaking, immunocomplexes were mixed with 10  $\mu$ L of 5 $\times$  sample buffer (50% glycerol, 0.1% Bromophenol Blue, 0.12 M Tris base), and aliquots were loaded onto 0.75% agarose gels made in running buffer (25 mM Tris, 19.2 mM glycine, pH 8.5). The gel was submerged in running buffer and electrophoresis was performed at a constant voltage of 50 V for 45 min at room temperature. Fluorescent bands were detected by illumination on a UV transilluminator operating at 303 nm. To quantify the fraction of QDs decorated with antibodies, we converted fluorescence images to gray scale and used Image J videodensitometric analysis to integrate the total fluorescence signal in lane 3 and in lane 4. We next integrated the fluorescence peak corresponding to antibody-free quantum dots and subtracted this result from total fluorescence measurements to obtain the amount of antibody-bound material. Results were

normalized to a total fluorescence of 1 to obtain values of 60% antibody decoration for BB-TrxA::CT43-stabilized nanocrystals and 70% for BB-CT43-stabilized nanocrystals. The experiment was repeated twice with similar results.

### **Analytical techniques**

UV-visible absorption spectra were recorded on a Beckman DU640 spectrophotometer. Fluorescence and phosphorescence emission spectra were recorded using 1 mL of sample on a Hitachi F4500 fluorescence spectrophotometer with excitation at 280 nm and excitation and emission slit widths set at 2.5 nm (fluorescence) or excitation at 316 nm and excitation and emission slit width at 2.5 nm and 10 nm, respectively (phosphorescence). The wavelength region corresponding to the second order diffraction peak of the excitation light was omitted. Hydrodynamic diameters and zeta potentials were measured on 1 mL samples using a Malvern Zetasizer Nano-ZS dynamic light scattering instrument equipped with a 633 nm laser filter. Data were fit with a Gaussian distribution to determine standard deviations.

- 1 W. Zhou, D. T. Schwartz and F. Baneyx, *J. Am. Chem. Soc.*, 2010, **132**, 4731.
- 2 W. Zhou and F. Baneyx, *ACS Nano*, 2011, **5**, 8013.