Supporting Information

Wide Dynamic Range Sensing with Single Quantum Dot Biosensors

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A. Stern-Volmer analysis of (dimethyl)aminomethylferrocene and CdSe@ZnS

Quenching of mercaptohexadecanoate-capped, ZnS-coated CdSe (CdSe@ZnS) nanoparticles with (dimethyl)aminomethylferrocene was examined. The titration was performed in pH 7.5 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer for comparison to other experiments. A solution containing ~100 nM CdSe@ZnS nanoparticle was examined by bulk fluorescence after 10 minutes of irradiation. Using a 100 μM stock of (dimethyl)aminomethylferrocene (Aldrich) in pH 7.5 MOPS buffer, successive additions were applied with a Hamilton syringe. The emission at 565 nm was monitored and corrected for small dilution effects. The emission response was plotted as (dimethyl)aminomethylferrocene concentration *verses* observed emission intensity divided by the initial emission intensity (I/I_o), shown in figure S1A. This analysis showed a Stern-Volmer quenching constant of $1 \bullet 10^7$ M^{-1}, suggesting a significant (dimethyl)aminomethylferrocene-CdSe@ZnS nanoparticle affinity under these conditions. Comparison of the the raw emission intensities (Fig. S1B) to the bulk emission response of **1**-appended MBP-attached CdSe@ZnS biosensor (Fig. 2, main text) suggests similar quenching for the free diffusing (dimethyl)aminomethylferrocene and MBP-attached ferrocene cases with a 1:1 stoichoimetry.

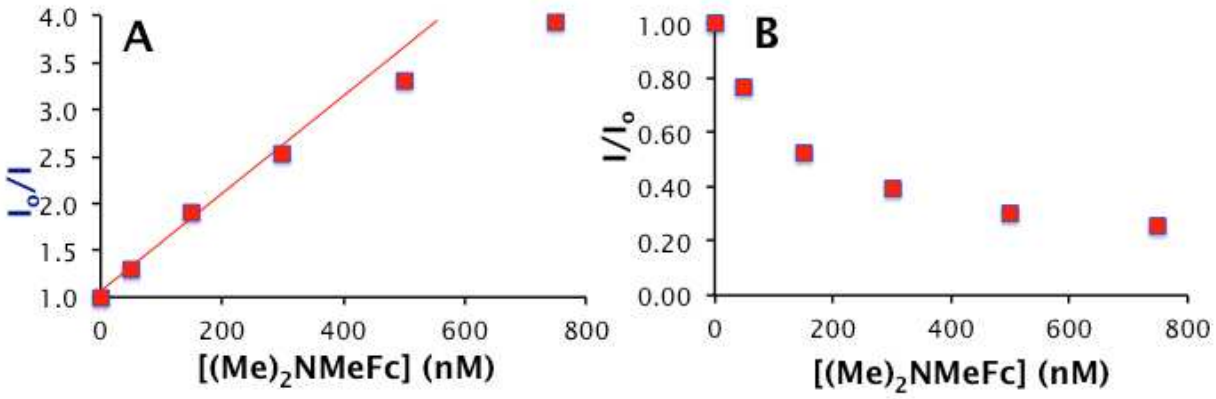

Figure S1. Quenching of CdSe@ZnS nanoparticles by (dimethyl)aminomethylferrocene ($(Me)_2NMeFc$). A) Quenching analysis displayed in Stern-Volmer formalism where the initial emission intensity (I_o) is divided by the emission intensity at a particular $(Me)_2NMeFc$) concentration (I). B) Quenching analysis reflecting the relative decrease in emission intensity (I/I_o).

B. pH dependence on single nanoparticle emission

Mercaptohexadecanoate (MHDA) capped CdSe@ZnS nanoparticles were assembled on the microscope cover slip for TIRF illumination as in the Methods section. After assembly the nanoparticles were washed with a pH 9.0 sodium borate (50 mM) solution for 2 minutes of flow followed by 5 minutes of rest. The area examined by TIRF microscopy was illuminated for 10 minutes prior to video collection. One video per pH was collected to minimize photolysis contributions. Single nanoparticles, traces, and emission intensities were determined as in the Methods section. After the pH 9.0 video was collected, nanoparticles were washed and examined as before with pH 8.0, 7.0, and 6.0 sodium phosphate (50 mM). Figure S1 shows the number of single nanoparticles (Fig. S1A) and the population average emission intensities (Fig. S1B). Since there were minimal changes in these metrics, single nanoparticle analysis is not shown. Clearly, minimal pH dependent changes in emission properties are shown for MHDA-capped CdSe@ZnS nanoparticles.

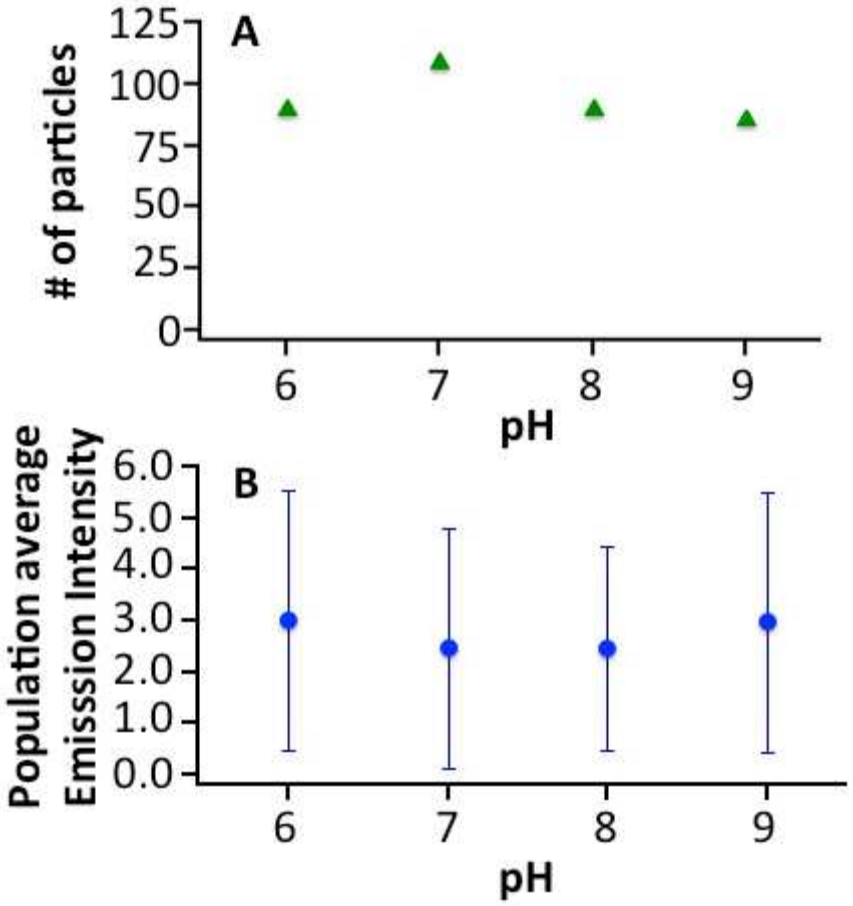

Figure S2. pH Dependence of Single Nanoparticle Emission Properties. A) Number of single nanoparticles identified from videos at different pHs from the same area of the slide. B) The population average emission intensities (error bars for population standard deviations) at various pHs for the same slide area.

C. Single nanoparticle emission over three hours

Full nanoparticle-based biosensors (**1**-MBP-CdSe@ZnS nanoparticle) were assembled on the microscope cover slip for TIRF illumination as in the Methods section. After assembly the nanoparticles were washed with a 50 mM tris(hydroxymethyl)aminomethane (Tris), 100 mM NaCl (pH 7.5) solution for 2 minutes of flow followed by 5 minutes of rest. The area examined by TIRF microscopy was illuminated for 10 minutes prior to video collection. Three videos per buffer addition were collected per buffer addition to simulate the maltose titration experiment (main text). Single nanoparticles, traces, and emission intensities were determined as in the Methods section. Each buffer addition included flowing 50 mM Tris (pH 7.5) 100 mM NaCl through the flow cell for 2 minutes followed b 5 minutes of arrested flow before video acqusition. Figure S2 shows the number of single nanoparticles (Fig. S2A) and the population average emission intensities (Fig. S2B). Since there were minimal changes in these metrics, single nanoparticle analysis is not shown. No significant changes in emission intensities or number of nanoparticles were observed over the three hour time course of this experiment.

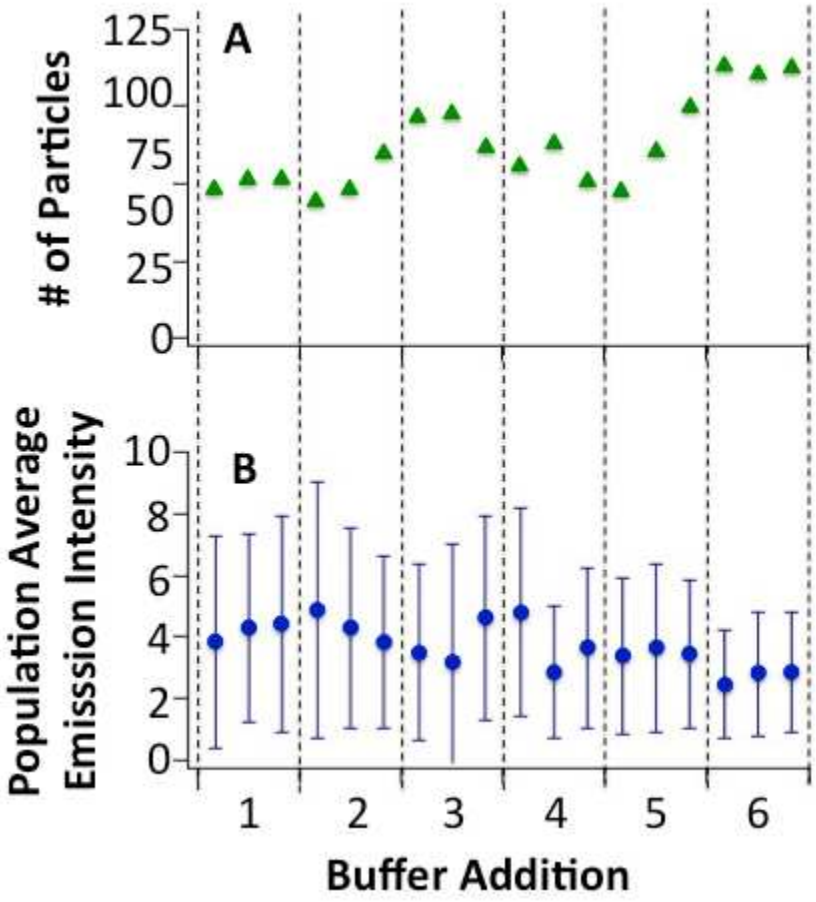

Figure S3. 50 mM tris (pH 7.5) 100 mM NaCl buffer addition to single nanoparticles over three hours. A) Number of single nanoparticles in each video. B) Population average intensity and standard deviation (error bars) from single nanoparticles identified from each video.

D. Maltose dependent response of single biosensor without ferrocene reductant

The nanoparticle-based biosensor assembly without the MBP-pendant ferrocene (**1**) was adsoped to the microscope cover slide as in the Methods section. Single particles were examined for any maltose-dependent response, as a negative control. A replicate of two conditions were examined: 50 mM tris (pH 7.5) 100 mM NaCl with 0 mM (Fig. S3, buffer) and 1 mM maltose (Fig. S3, maltose). Each condition was provided by 2 minutes of solution flow followed by 5 minutes of arrested flow before video acquisition. The initial acquisition (first buffer addition) was collected after 10 minutes of illumination. Figure S3 shows the number of single nanoparticles (Fig. S3A) and the population average intensities of the single nanoparticles (Fig. S3B) determined from these movies (3 per condition). The overall emission metrics did not change for these populations upon adding saturated maltose when the MBP-pendant ferrocene (1) was not included in the assembly.

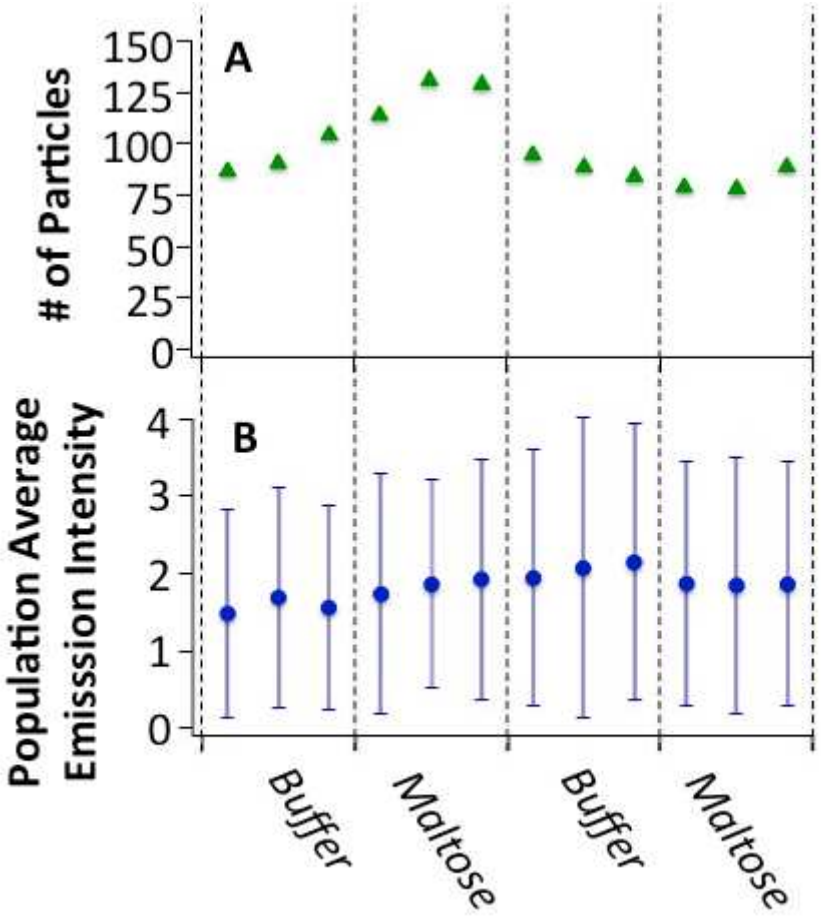

Figure S4. Maltose dependent emission intensity of single biosensors without MBP-pendent ferrocene (**1**): A) Number of single nanoparticles determined for each movie of the same area; B) Population average emission intensities (error bars, population standard deviation) of single nanoparticles from each movie. Two conditions were re-peatedly examined: 50 mM tris (pH 7.5) 100 mM NaCl with 0 mM (Buffer) and 1 mM maltose (Maltose).

E. Electrophoretic analysis of MBP to CdSe@ZnS nanoparticle stoichiometry.

The stoichiometry of MBP-His_6 per MHDA-capped CdSe@ZnS nanoparticle samples was determined by agarose electrophoresis. Bioconjugate samples, prior to bulk or single molecule fluorescence analysis, (~ 500 nM) were mixed with 14% glycerol (1:1) and loaded (~10 μL) into the wells of an 1% w/v agarose gel. The agarose gel was cast from a 1x TAE buffer (40 mM tris(hydroxyethyl)aminomethane, 40 mM acetic acid, 1 mM ethylenediaminetetraacetic acid, pH 8.5), which was also used as the running buffer. Wells containing the samples were on the side of the negative electrode and the samples were draw into and separated by being pulled towards the positive electrode (70 V constant voltage for 1 hour). The overall negative charge from the CdSe@ZnS nanoparticle surface and MHDA capping groups at this pH facilitated sample migration through the gel and separation based on size of the nanoparticle. Therefore, a sample with a higher molecular weight will migrate slower than a lower molecular weight. Stoichometry is derived by migration relative to MHDA-capped CdSe@ZnS nanoparticles and the peak width of a sample relative to the peak width of MHDA-capped CdSe@ZnS nanoparticles. Figure S5 shows a 1:1 MBP to nanoparticle stoichiometry for a typical sample (lane 2); from a decreased migration relative to MHDA-capped CdSe@ZnS nanoparticles alone and a similar peak width between the two samples.

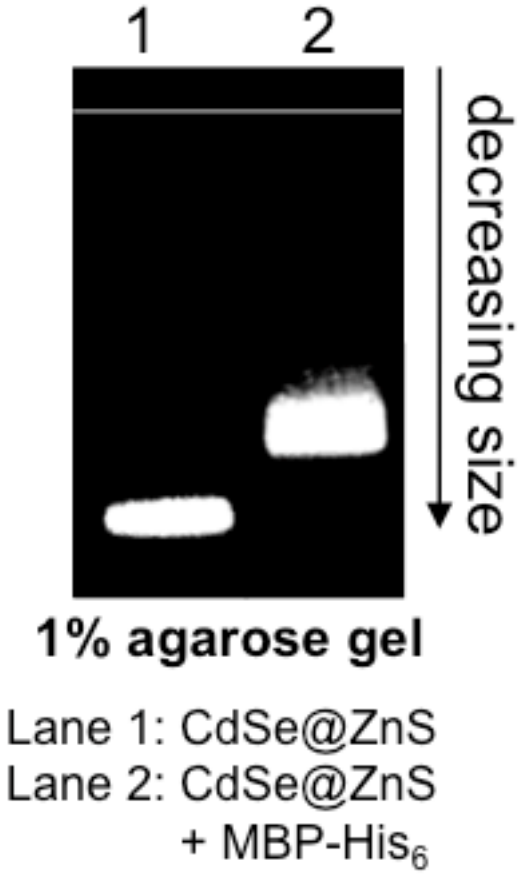

Figure S5. Electrophoretic analysis of MBP to CdSe@ZnS nanoparticle stoichiometry. Nanoparticle samples (500 nM) were mixed 1:1 with 14% glycerol and loaded in 1% agarose gels. Samples were ran towards a positive electrode (70 V, constant power) and images using the intrinsic fluorescence of CdSe@ZnS nanoparticles. Lane 1, MHDA-capped CdSe@ZnS nanoparticles; Lane 2, MBP-His_6 attached, MHDA-capped CdSe@ZnS nanoparticles.