Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Engineering quantum dot calibration beads standards for quantitative fluorescence profiling

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Supporting information:

Quantum dots ICP-MS quantification analysis

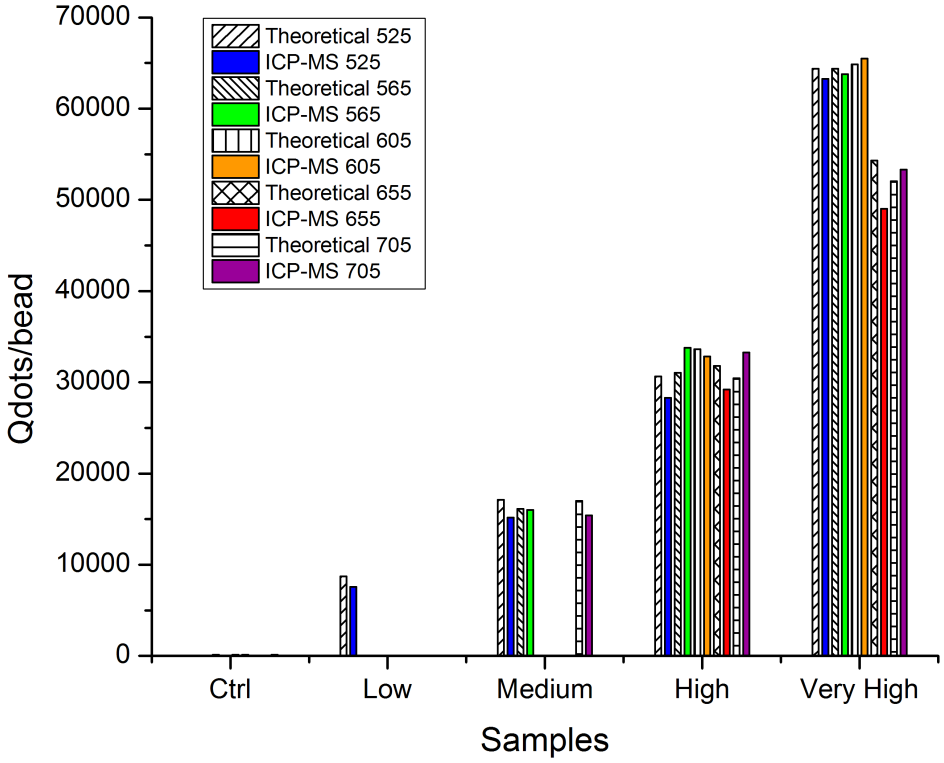

Figure S1. Comparison of ICP-MS quantification analysis and theoretical values of Qdots on beads for 525, 565, 605, 655, 705 nm Qdots.

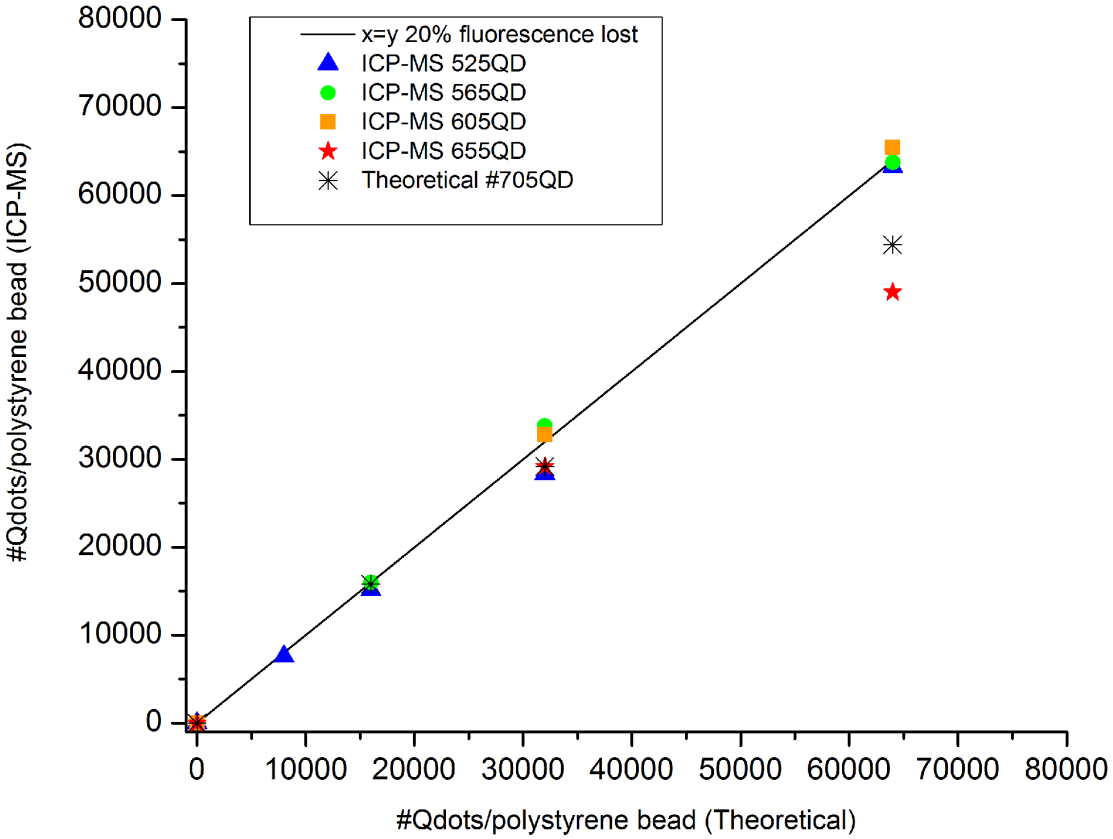

Figure S2. Comparing a 20% linear fluorescence loss in all samples with the actual ICP-MS Qdot estimation values.

Biotin polystyrene beads: 1% w/v 6.0-8.0 µm
Average diameter: 6.8 µm
Binding capacity: 867 ng of avidin-FITC to 1 mg of particle
Concentration: ~5790 microspheres per µl
Number of streptavidin FITC binding sites/bead: $1.5e^{6}$

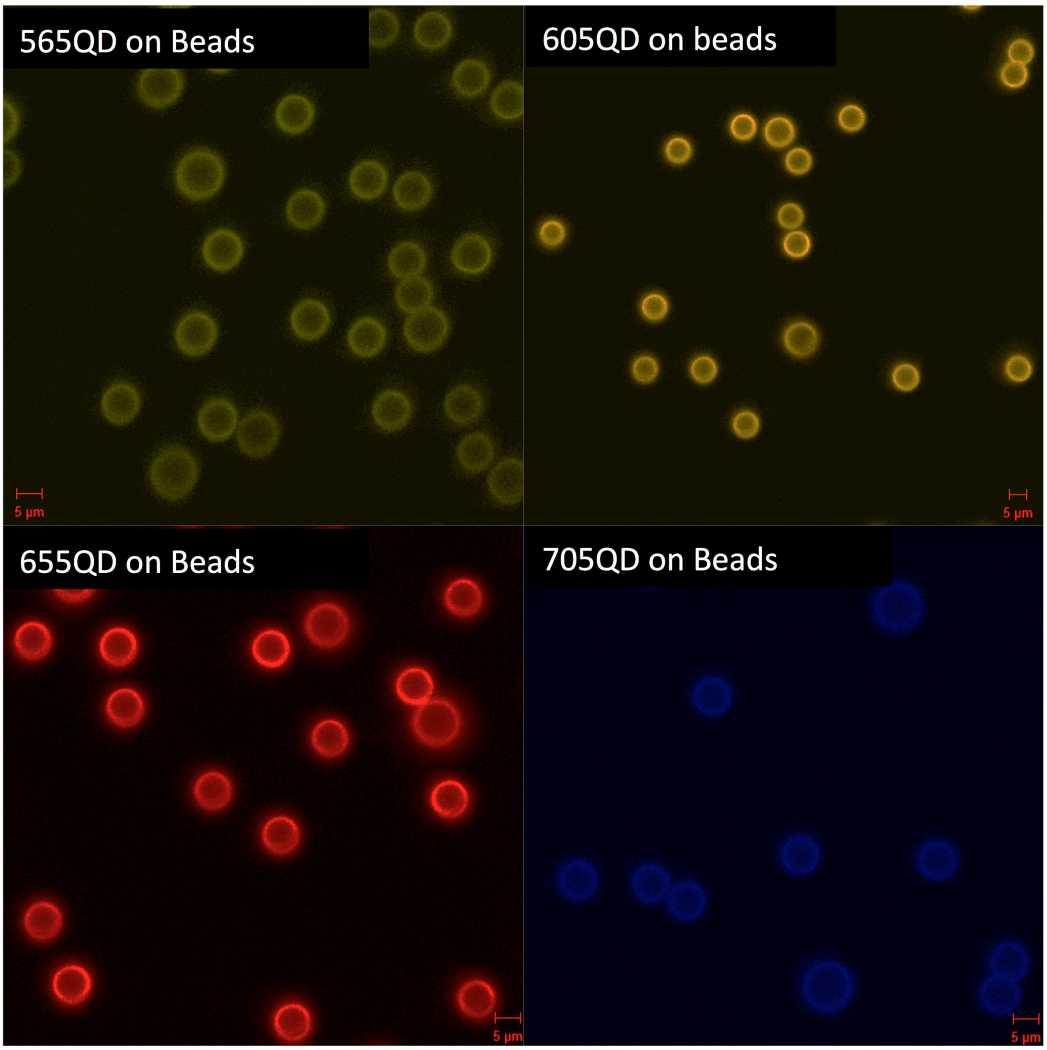

Figure S3. Confocal images of Qdots on polystyrene beads. Yellow (565 SAv-Qdots), Orange (605 SAv-Qdots), Red (655 SAv-Qdots) and Blue (705 SAv-Qdots).

Effects of pH and BSA on Qdots

The streptavidin-biotin complex is the strongest known non-covalent biological interaction between protein and ligand; it forms rapidly and is stable, being resistant to a wide range of pH, temperatures, organic solvents and other denaturing agents. For example, the biotin avidin complex is not significantly affected by pH between 2-13, nor by concentrations of guanidinium chloride up to 8M at neutral pH [1-3]. In addition, biotin binding increases the midpoint temperature of thermally-induced denaturation of strevtavidin and avidin in phosphate buffer from 75°C and 83°C to 112°C and 117°C at full biotin saturation, respectively. This makes the Qdot calibration beads stable over a longer period of time [4]. Consistent with this stability, we havae observed that the Qdots were stable for at least five months under room temperature conditions.

We tested the effect of 3 different pH phosphate buffered solutions (pH 4, 5 and 7.4) on the storage our Qdot conjugated beads. Our results suggest some problems with the Streptavidin-Qdots following long-term storage (more than one year). Long-term storage of Qdots can cause some aggreagation over time and also potential degradation of streptavidin if contaminated with microorganisms. The results in Figure S4 show a decrease in fluorescence for all pH PBS buffers, however we are concern in the lost of fluorescence when we used pH 7.4 after the washing steps compared to previous fluorescence values.

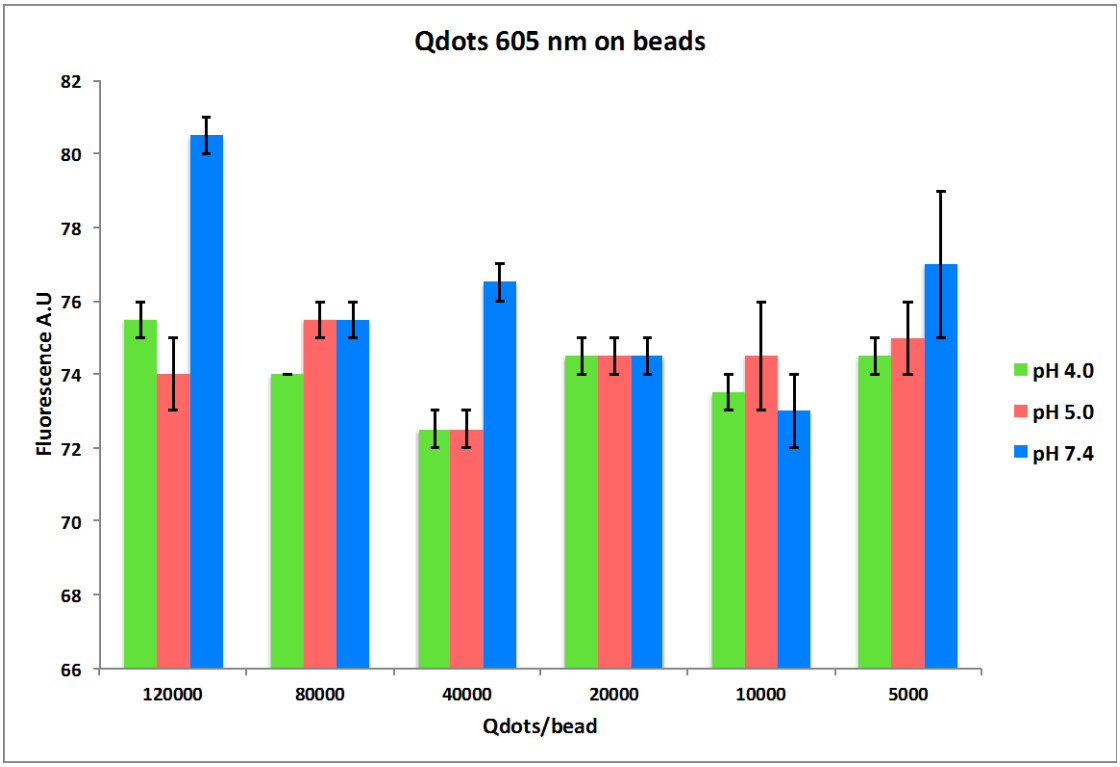

Figure S4. pH effects on Qdot 605 conjugated with biotynilated polystyrene beads.

Figure S5 shows a clear effect of the lower pH on the fluorescence intensity of Qdots. At pH 4.0 there is approximately a 25% reduction in fluorescence and at pH 5.0 close to a 20% reduction, compared to pH 7.4.

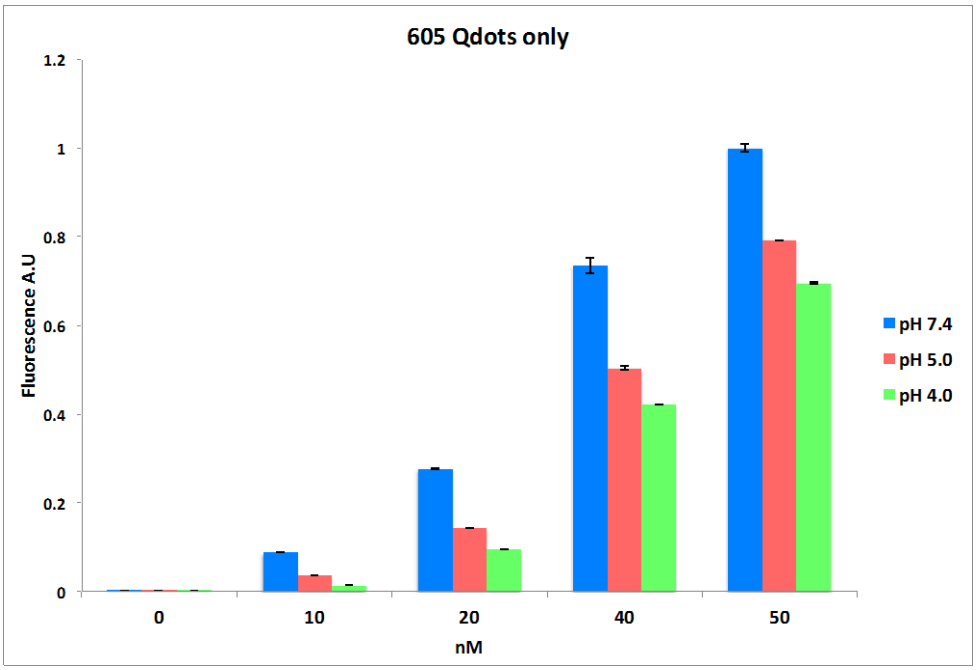

Figure S5. Normalized fluorescence intensity of Qdots 605 nm after exposure to different pH levels.

We tested the effects of bovine serum albumin on the fluorescene intensity of Qdots 655 nm. After 18 h of incubation there was a singnificant quenching effect on the fluorescence of the Qdots (Figure S6).

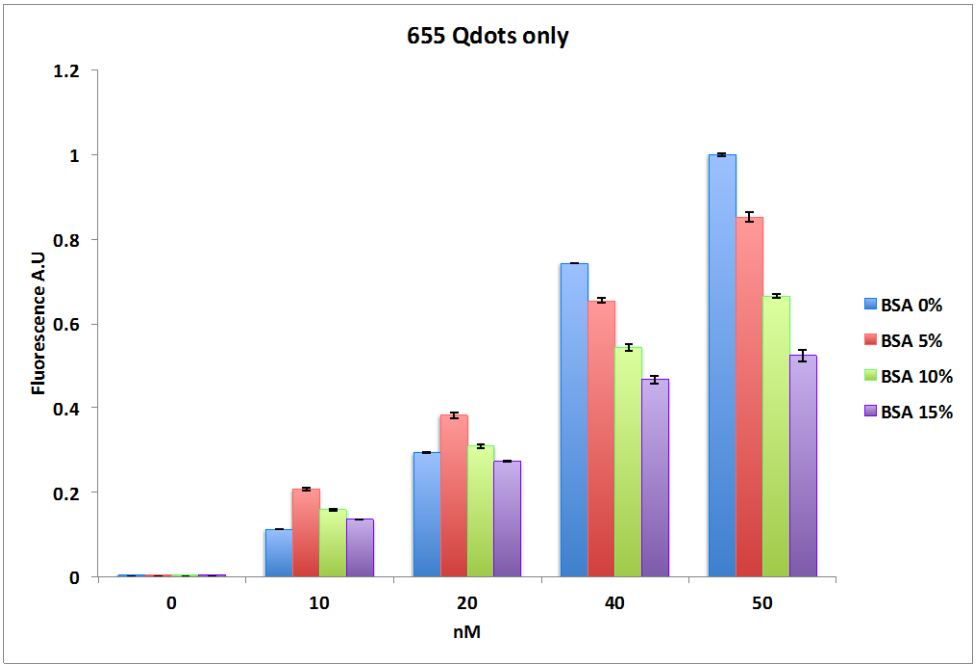

Figure S6. Effects of 3 different concentrations of BSA in PBS on the fluorescence intensity of 655 nm Qdots.

References

1. M. D. Savage, in *A Laboratory Guide to Biotin-Labeling in Biomolecule Analysis*, eds. T. Meier and F. Fahrenholz, Birkhäuser Basel, 1995, pp. 1-29.
2. M. D. Savage, *Avidin-Biotin Chemistry A Handbook, Pierce nd Warriner*, 1992.
3. N. M. GREEN, *Biochem. J.*, 1963, **89**, 599-590.
4. M. n. González, C. E. Argaraña and G. D. Fidelio, *Biomolecular Engineering*, 1999, **16**, 67-72.