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Supporting Information

Addressing Key Technical Aspects of Quantum Dot Probe Preparation for Bioassays

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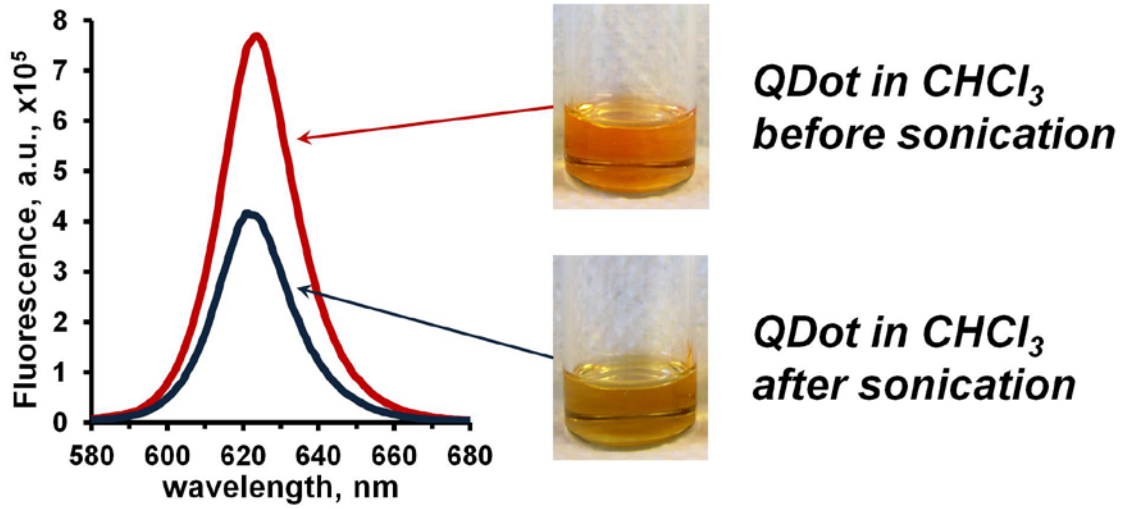

Figure S1. Degradation of hydrophobic QDots by bath sonication. Hydrophobic nanoparticles dissolved in a non-polar solvent (such as chloroform) are stabilized by labile hydrophobic surface ligands, which are essential for QDot colloidal stability and optical properties. Depletion of surface ligands might expose “charge traps” on the nanoparticle surface, leading to dramatic quenching of fluorescence and potential degradation of the nanoparticle core via interaction with an environment. In particular, bath sonication, commonly employed for suspending and mixing QDots, proved highly damaging for hydrophobic QDots dissolved in chloroform, leading to almost 50% drop in fluorescence intensity even after a brief exposure. Therefore, any harsh treatment should be avoided at all steps of polymer encapsulation procedure, and only mild shaking can be employed for mixing hydrophobic QDots. Notably, polymer-coated QDots exhibited outstanding stability in a range of conditions and could be safely mixed by sonication without particle degradation.

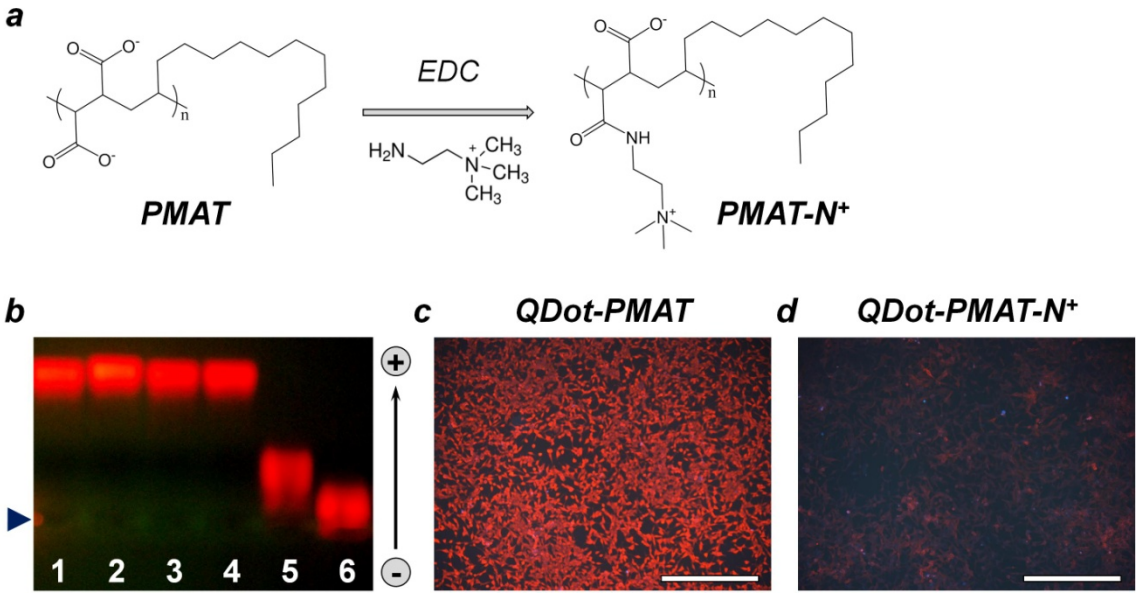

Figure S2. Surface modification of PMAT-coated QDots for reduced non-specific binding to biological specimens. (a) Schematic of PMAT modification with positively-charged trimethylammonium groups. Partial substitution of carboxylic acid groups by $–N^+$ effectively reduced overall surface charge, producing a highly hydrophilic zwitterionic surface, while preserving the original particle size. (b) Agarose gel electrophoresis demonstrated reduced motility of $–N^+$ modified QDots (lane 5 and 6, corresponding to 10,000x and 100,000x molar excess $–N^+$ to QDot used in a reaction) in comparison to unmodified PMAT-coated QDots (lane 1) and control QDots mixed only with EDC (lane 2), 10kx $–N^+$ (lane 3), and 100kx $–N^+$ (lane 4). Interestingly, no positively-charged QDots could be produced with this procedure, likely due to steric hindrance between trimethylammonium groups, which prevented modification of more than 50% of carboxylic acid groups. (c) When incubated with cultured LNCap cells fixed with formalin and permeabilized with detergents, as routinely done for labeling of intracellular targets, PMAT-coated QDots produced very high non-specific staining due to electrostatic interaction between its negatively-charged surface and the specimen. In contrast, same particles modified by $–N^+$ and featuring a nearly neutral surface charge produced a dramatically lower non-specific labeling (d), which could be further reduced by blocking the specimen with bovine serum albumin or casein. Scale bar, 500 µm.

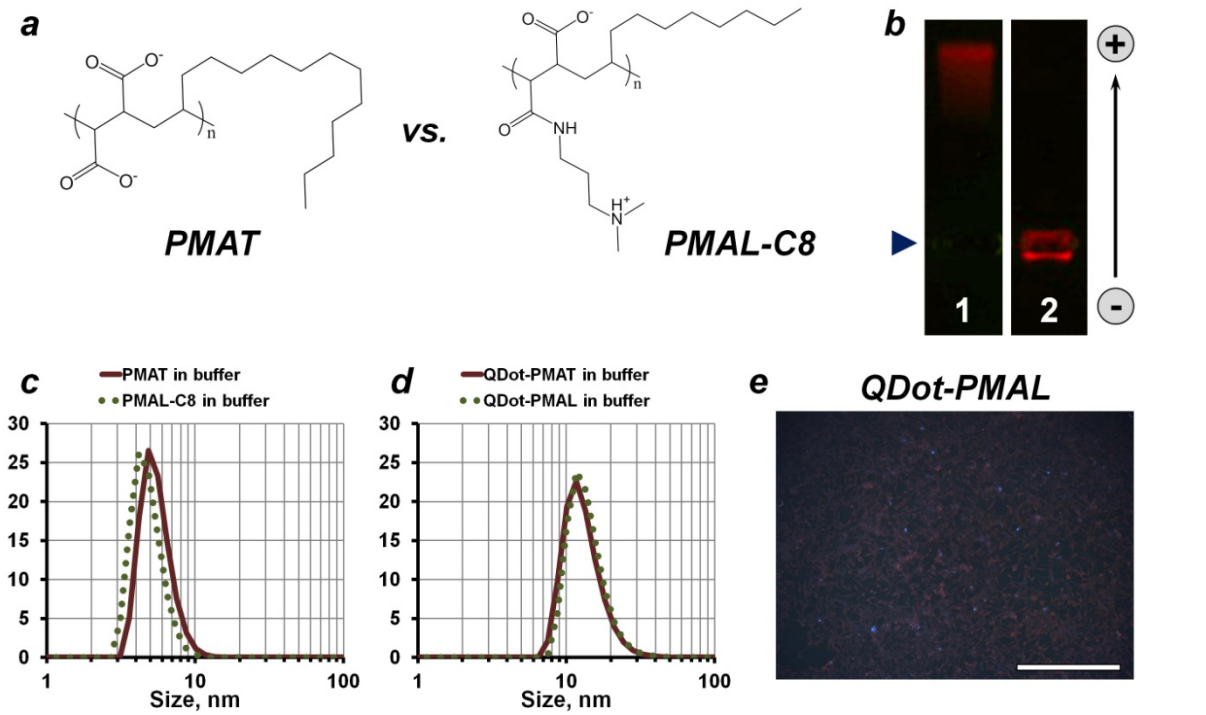

Figure S3. Encapsulation of QDots with zwitterionic polymers. (a) Schematic of negatively-charged PMAT and zwitterionic PMAL-C8 polymers. (b) In contrast to QDot-PMAT (lane 1), PMAL-coated QDots lacked an overall surface charge, showing lack of migration in agarose gel electrophoresis (lane 2). Loading well position is indicated by blue arrowhead on the left. (c-d) At the same time, both polymers share same backbone structure, similar aliphatic side-chains, and similar molecular weight, forming similarly-sized micelles in an aqueous buffer (c) and, thus, producing similarly-sized hydrophilic polymer-coated QDots upon encapsulation procedure (d). (e) Lack of an overall surface charge effectively prevented non-specific QDot binding to cultured LNCap cells fixed with formalin and permeabilized with detergents, making such particles a suitable platform for further development of fluorescent probes for intracellular labeling. Scale bar, 500 µm.