

Supporting Information
Immunoassay Based on a Photoluminescence Quenching of the
Antibody-Conjugated PAMAM-Dendrimer–Gold Quantum Dot Complex

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Materials and methods

Synthesis and conjugation of gold QDs. Gold QDs with an emission maximum at 450 nm were prepared according to methods previously reported in the literature^{3,4}: 0.5 μmol G2-OH (Sigma-Aldrich) and 1.5 μmol $\text{HAuCl}_4 \cdot \text{nH}_2\text{O}$ (Sigma-Aldrich) were co-dissolved in 2 mL of distilled water (resistivity 18 $\text{M}\Omega\cdot\text{cm}$ and surface tension of 72.6 mN/M at 20.0°C, Continental Modulab 2020). The gold ions were sequestered within the dendrimer by allowing 30 min of stirring prior to the slow addition of an equivalent of the reductant NaBH_4 (Sigma-Aldrich). The solution was stirred for 2 days in order for reaction and aggregation processes to complete. The resulting solution was then purified through ultracentrifugation at 16000 g for 45 min (Beckman L7 Ultracentrifuge) resulting in separation of the larger Au nanoparticles from the smaller Au QDs, leaving a clear solution. Au QDs were then suspended in 2 mL PBS buffer (pH 6.3) and mixed with 1 mg of polyclonal antibody to human IgG (goat affinity purified antibody to human IgG; MP Biomedicals). The antibody-dendrimer-Au-QD complex was conjugated electrostatically at the above mentioned pH. The solution was vortexed for 12 h. All of the work involving immunocomplexation of antibody-QD complex with recombinant human IgG antigen was completed in the presence of PBS buffer at a pH of 6.3. As stated, it was previously reported⁴ that AuQDs of varying sizes, and thus emission maxima, are able to be synthesized using this method. However, we were unable to reproduce this finding: only AuQDs with emission at 450 nm were able to be synthesized.

Nanoscale imaging of particles, antibody-particle conjugates, and antigen-conjugate complex. For the purpose of visualization of the structure and topography of gold nanoparticles, Au nanoparticle- Anti-Human IgG polyclonal antibody conjugates, and immunocomplex formed after addition of recombinant human IgG antigen, we have utilized high-resolution transmission electron microscopy (HRTEM). All of the high-resolution TEM imaging was done on the Philips CM 20 TEM with LaB6 electron gun and EDS (EDAX/4pi) system. The system has accelerating voltage of 200 kV with image point resolution of 0.278 nm, line resolution of 0.14 nm and maximum magnification of 750,000 X in the high-resolution mode. The diluted sample was drop-

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coated on the surface of lacey carbon grid, let dry and introduced into the vacuum chamber.

For the purpose of topographic characterization, an atomic force microscopy (AFM), measurements were performed on the Dimension 5000 AFM microscope (Veeco Instruments Inc, Santa Barbara, CA), in the tapping mode. Imaging was conducted with a sharp silicon cantilever and under a moderately-slow scan, with a scanning frequency of 1.508 Hz.

Photoluminescence characterization was performed on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ) while absorption measurements were performed on a Perkin Elmer Lambda 900 UV/Vis/NIR spectrometer. Sample cells used for both measurements were composed of quartz and had an optical pathlength of 1 cm.

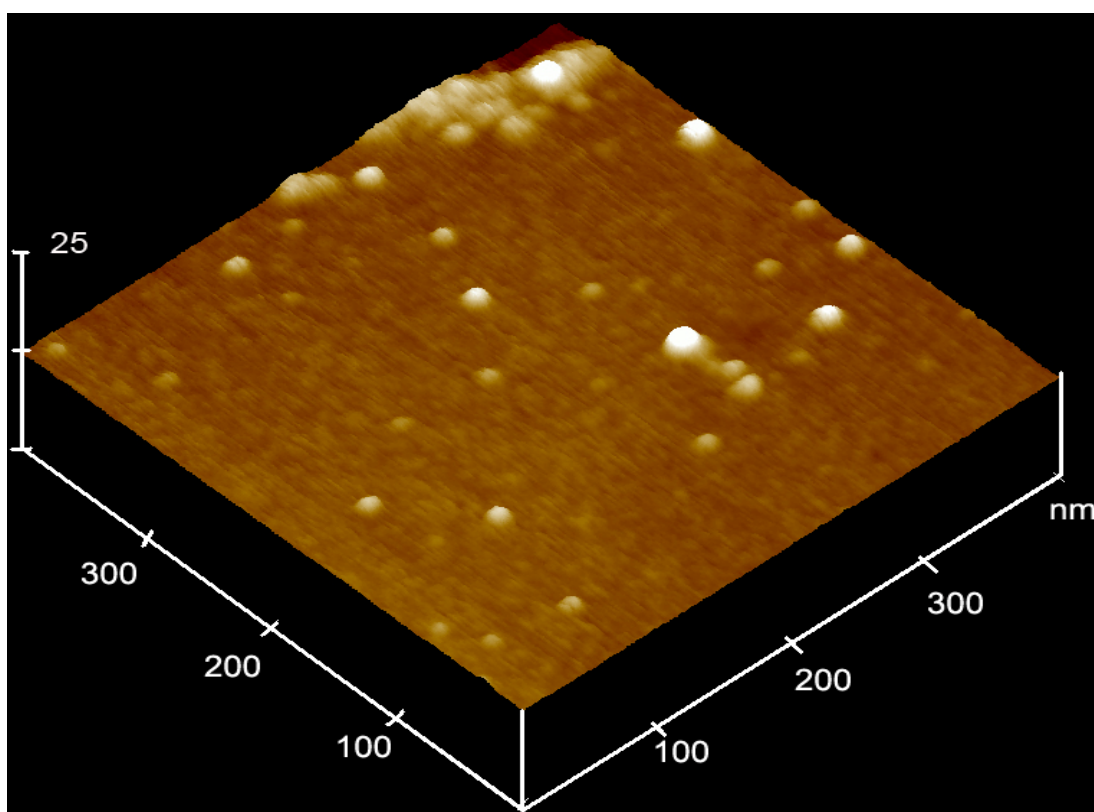


Figure S1. AFM micrography of gold quantum dots, image size 400X400 nm.

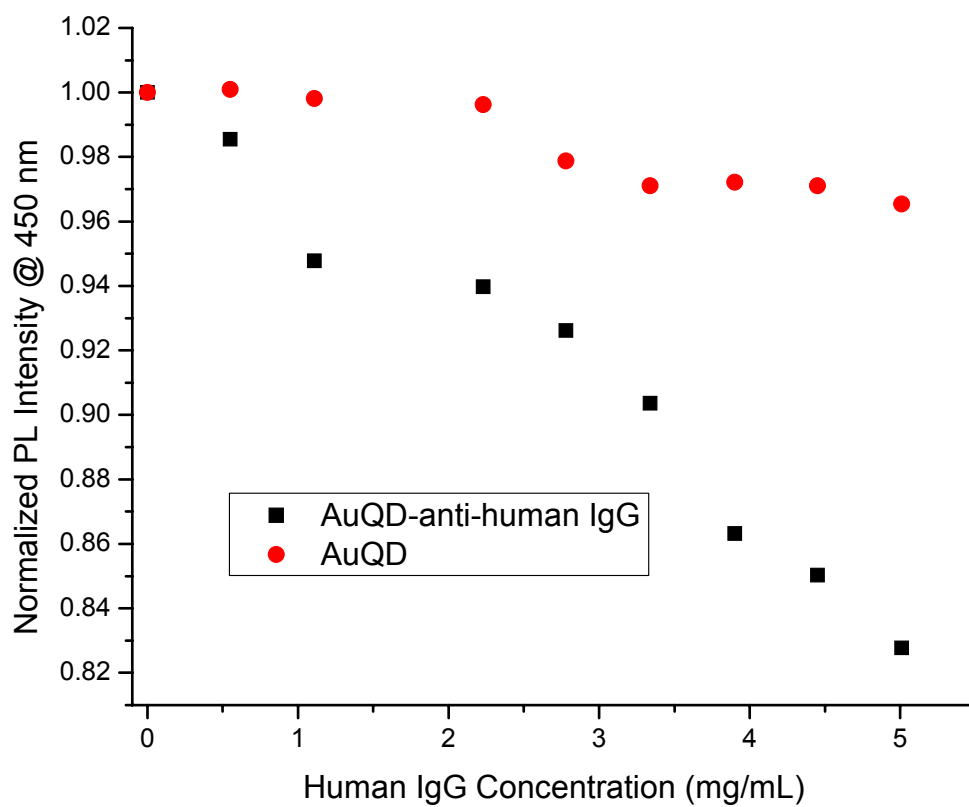


Figure S2. Normalized fluorescence intensity at 450 nm for AuQD (●) and AuQD-anti-human IgG (■) versus human IgG concentration.

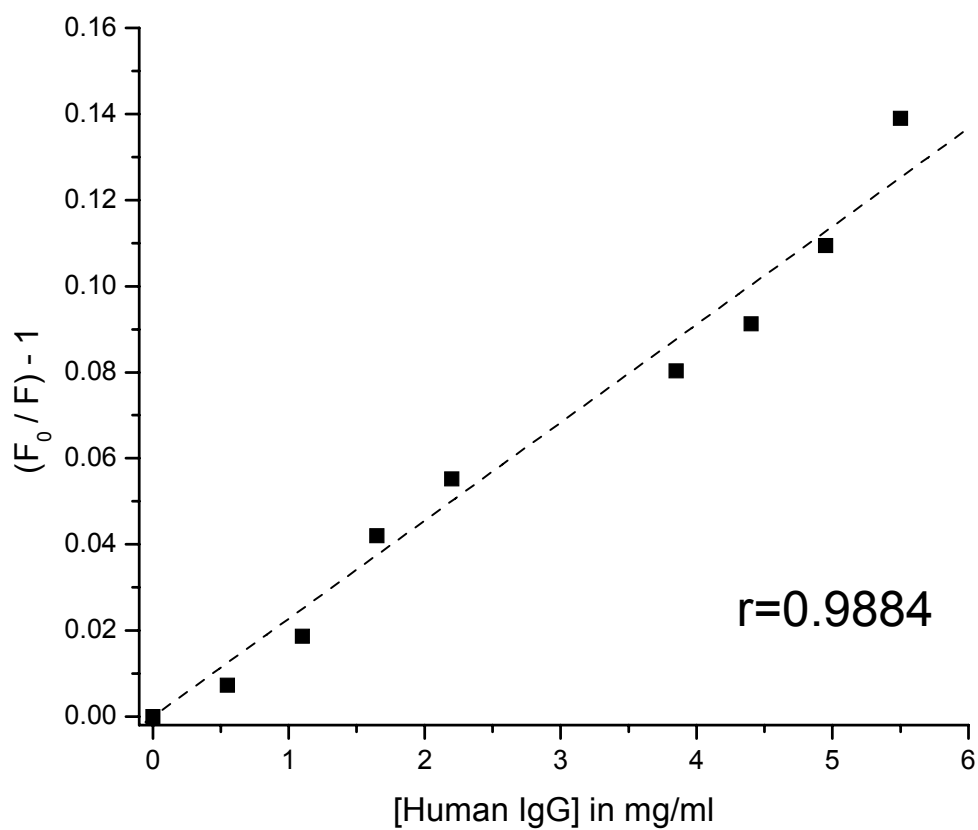


Figure S3. Stern-Volmer plot of the AuQD-anti-human IgG complex titrated with human IgG.