Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Preferential interactions of primary amine-terminated quantum dots with membrane domain boundaries and lipid rafts revealed with nanometer resolution

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SUPPLEMENTAL METHODS

Cell Viability Assay. The impact of Qdots705 on trout gill epithelial cell viability was assessed using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) (Promega, G3580). Cells were seeded in 96-well plates at 50,000 cells/well in 200 µL of complete media. Following 100% confluence, cells were rinsed 1× with media, and exposed to 100 µL of Qdots at various concentration (0-50 nM) in media for 24 hrs. For a negative control, cells were exposed to lysis buffer (Promega) for 30 min before the end of the Qdot exposure. After 24 hr, cells were rinsed 3× with phenol-red free media, and incubated with 20 µL of MTS solution in 100 µL of phenol-red free media for 4 h at 19° C. The absorbance was read at 490 nm using a Molecular Devices SpectraMax Plus 384 Multi-mode Microplate Reader.

Calculate the number of amine groups on the Qdot surface. Nuclear magnetic resonance (NMR) was used to quantify the total concentration of primary amine moiety in the Qdot605 sample. All measurements were performed using a Bruker Avance-600 spectrometer with TCI-F cryoprobe. The recycling time (d1) was always 3-5 times longer than the longest T_1 of interest and all integrated areas were normalized by the number of scans and the receiver gain. The NMR measurements showed a surface coverage of 107.8 amines per Qdot, as described in detail under **Figure S3** (below). Furthermore, the surface area of the Qdot was measured using Brunauer-Emmett-Teller (BET). BET-specific surface area of the Qdot was determined from N_2 adsorption/desorption isotherms obtained using a Micromeritics Gemini VII 2390 surface area analyzer. The Qdot sample was dried at 25°C under vacuum overnight. The sample was subsequently introduced into the analyzer and measured over the relative pressure range (P/P_0) of 0.05-0.3, where P_0 is the saturated pressure of N_2. The BET measurement showed a surface area of $1.62 \cdot 10^{16}$ $nm^2 \cdot mg^{-1}$ and these values yield a surface amine density of 0.1 amine per nm^2.

SUPPLEMENTAL FIGURES

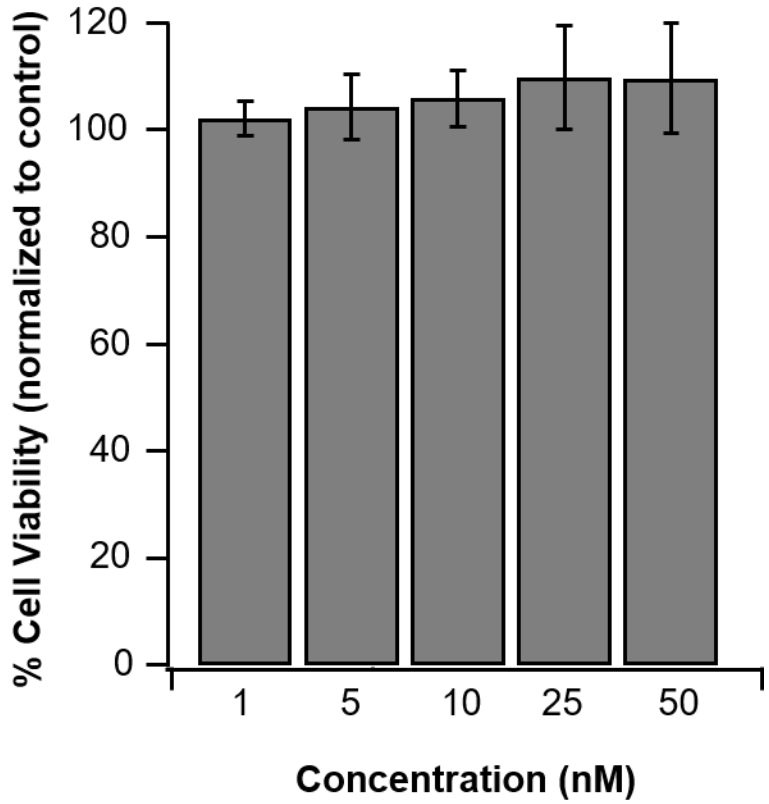

Figure S1. Gill epithelial cells exposed to primary amine-terminated Qdots for 24 h show no decrease in cell viability at concentrations up to 50 nM, as determined by the MTS assay.

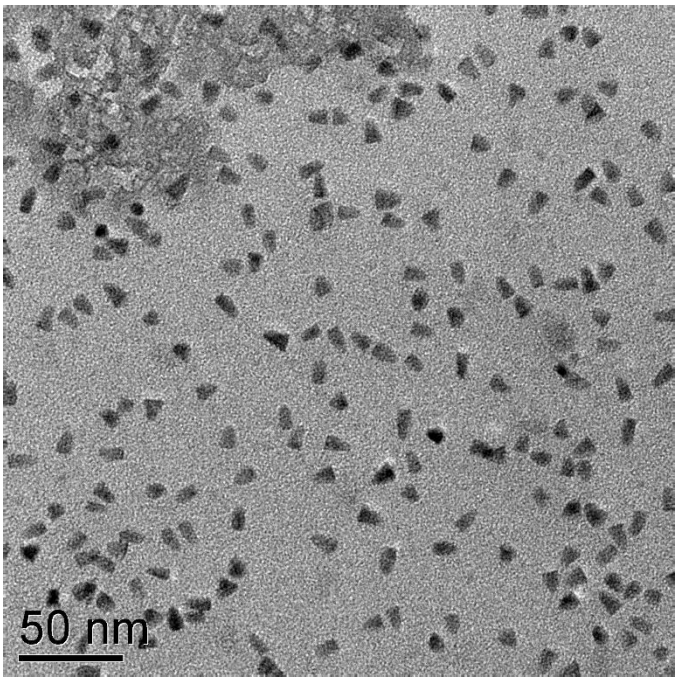

Figure S2. TEM image of Qdots705 used for cell imaging, diluted in cell culture medium. The Qdots appear as individual particles under this solution condition. Cluster containing salt and other culture medium ingredients is visible on the upper left corner.

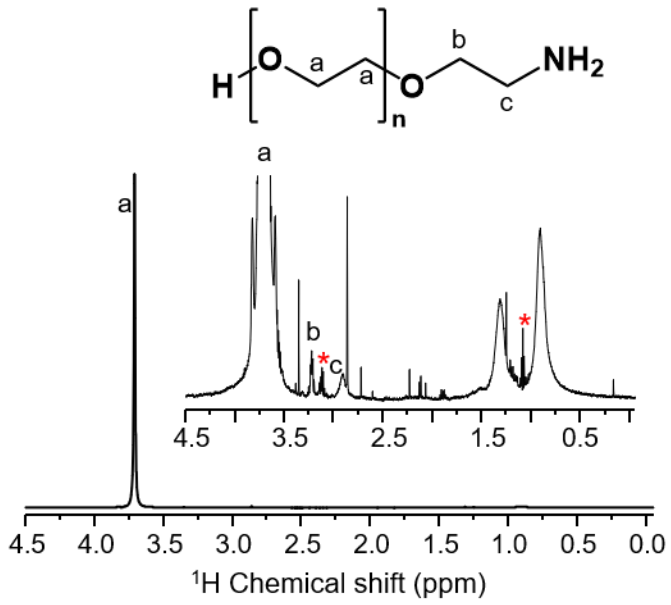

Figure S3. The quantitative ^{1}H-NMR spectrum of the functionalized Qdot605 in D_2O. The insert window shows the zoom-in region between 0.5 ppm and 4.5 ppm. The red asterisk corresponds to residual ethanol. Five broad peaks are found in the spectrum. Five broad peaks can be seen that are associated with the polymeric functionalization at the Q-dot surfaces, with trace impurities (all other small peaks in the spectrum). The two broad peaks at 0.9 ppm and 1.3 ppm correspond to protons from the poly acrylic acid that is used as molecular linker between the Qdot surface and the amine-hydroxyl PEG ligand. The broad peak at 2.9 ppm corresponds to protons that are attached to the carbon adjacent to the primary amine head group (labeled as c) and is used to quantify the concentration of the overall amine moiety in the samples. The broad peak at 3.2 ppm and 3.7 ppm are associated with the PEG moiety (labeled as a and b). By integrating the area under the peak at 2.9 ppm and comparing it to control samples with known concentrations, our results show a total amine concentration of 27.8 µM ± 1.4 µM (5% error) for 0.3 µM of Qdot (9.6×10^{13} Q-dot particles). These results translate to a surface coverage of 107.8 amines per Q-dot particle.